Juvenility, Puberty and Adolescence Among Bangladeshi and British Youth

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JUVENILITY, PUBERTY AND ADOLESCENCE AMONG BANGLADESHI AND BRITISH YOUTH

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MATERIAL ABSTRACT

The ABBY (Adolescence among Bangladeshi and British Youth) Project explores the relationship between migration and growing up from a biocultural perspective. Based on evolutionary hypotheses, it tests for facultative adaptation to different developmental environments during the transition from child to adolescent using contrasting conditions within ethnicity, ecology, and migration. I explore the relationship between these variables and the timing and tempo of adrenarche, thelarche, pubarche and menarche through comparisons of biological and cultural markers of development among 488 girls, aged 5–16, belonging to the following groups: Sylheti, first generation British-Bangladeshi, second generation British-Bangladeshi and white British. This project supports evidence that the timing, tempo and experience of juvenile and pubertal development vary across populations with possible lasting implications for the strategic allocation of reproductive effort. Specifically, adrenarche occurred two years earlier in first generation migrant girls to Britain, suggesting that change in ecological factors results in more rapid juvenile onset. Thelarche occurred earlier with increasing individual and ancestral generations lived in the UK, suggesting that local ecological factors result in earlier pubertal onset. Contrary to predictions, menarcheal timing and oestrogen levels did not differ significantly among groups. Acculturation did not account for differences in behaviours during juvenile and pubertal development between groups. Instead, the stages of practising to being dedicated to hijab (which occur during juvenility and after puberty, respectively) better reflect the social process of growing-up as Bangladeshi girls in East London. Growing up here may be uniquely stressful among first generation migrants. Psychosocial stress may interact with other ecological factors resulting in an overall slower tempo of juvenile development. The extended period of plasticity during juvenility among girls who experienced a change in socio-ecological factors may be an adaptive response to ensure a better tracking of current socio-ecological conditions and also a better prediction of later ones.

JUVENILITY, PUBERTY AND ADOLESCENCE AMONG BANGLADESHI AND BRITISH YOUTH

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Thesis submitted for the degree of
Doctor of Philosophy
to
Department of Anthropology
Durham University
2013

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List of Abbreviations and Terms

ABBY: The ABBY Project (Adolescence among Bangladeshi and British Youth)

Adrenarche: the rise in adrenal androgen production; the beginning of juvenility; the puberty of the Hypothalamic-pituitary-adrenal (HPA) axis

Bangladeshi: all girls of Bangladeshi ethnicity

British-Bangladeshi: Bangladeshi migrant groups living in the UK

DHEAS: Dehydroepiandrosterone-sulfate

Oestrogen (Total): Sum of all 15 oestrogen and oestrogen metabolites measured in urine

Energetic Stress: stress induced by nutritional, physical or immunological factors

First generation: same as British-Bangladeshi migrants

Freshi: British- Bangladeshi term used for first generation migrants

Gonadarche: in girls, the activation of the Hypothalamic-pituitary-ovarian (HPO) axis which includes thelarche, pubarche and menarche

IAG: Individual/ancestral generations lived in the UK; used in reference to migration scale

Juvenility/Juvenile period: the stage in the human life course between childhood and adolescence

Juvenile secondary sex characteristics: those physical characteristics that occur during the juvenile period including: axillary hair, lower leg hair, body odour, pimples, oily skin

Londoni: Sylheti term which refers to British-Bangladeshis living in the UK

Migrants: girls born in Sylhet who migrated to the UK in their lifetime

Migrant groups: migrants and their descendants living in the UK

Migrant status: distinction between migrants and the subsequent generations of their descendants

Migration scale: distinction between Sylheti, first generation, second generation and white British girls; dummy variable based on individual/ancestral generations (IAG) live in the UK. The IAG values for each migration group are: Sylhetis = 0, first generation=1, second generation=2, white British = 3.

Pubarche: the onset of pubic hair growth

Pubertal secondary sex characteristics: those physical characteristics that occur during the pubertal transition including: pubic hair and breast development

Pubertal onset: the first signs of puberty; usually marked by thelarche or pubarche

Puberty: the transition between juvenility and adolescence; synonymous with gonadarche which in girls, the activation of the Hypothalamic-pituitary-ovarian axis which includes thelarche, pubarche and menarche

Psychosocial stressor: stress responses induced by social and psychological factors

SSC: Secondary sex characteristics

Second generation: Bangladeshi girls born and living in the UK

Sylheti: girls born and living in Sylhet, Bangladesh

Thelarche: the onset of breast development

White British: girls born and living in the UK of European ethnicity

Stress: the response of an organism to its environment (natural or social) in ways that prepare it to deal with the immediate allocation of energy and perceived or real threats to the organism's survival and reproduction

DECLARATION

I, Lauren Claire Houghton, declare that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

STATEMENT OF COPYRIGHT

ACKNOWLEDGEMENTS

It takes a village to raise a child; people in many villages helped to raise ABBY. I express my fullest gratitude to the participants and their families, without their generosity of time and trust I would have no thesis to share. I would like to acknowledge the numerous school administrators, teachers and assistants that opened their classrooms in East London and Sylhet to the ABBY Project. I acknowledge the funding that has made my education and research possible which was provided by the NIH-Wellcome Trust Fellowship programme and the Division of Cancer Epidemiology and Genetics at the National Cancer Institute.

In Durham, I thank: Professor Gillian Bentley whose perceptive supervision and profound expertise of reproductive ecology has inspired me to pursue a rewarding research trajectory; Dr Mark Booth for showing me how to discern what really matters as a researcher, professional and global citizen; Dr Kate Hampshire for teaching me through example how to listen, observe, engage and deconstruct in the construction of this thesis; Dr Gill Cooper for completing all salivary assays and for being a caring collaborator whether in the lab, at the desk or over the phone. I also thank Dr Mwenza Blell, Rasha Chaklader, Dr Emilie Fairet, Dr Carla Handley, Dr Caroline Jones, Dr Kristin Klingaman, Dr Rebecca Langford, Dr Hayley Lofink, Vicki McGowan, Savita Sathe, Megan Wainwright and Aurang Zeb for being the incredibly intelligent colleagues that they are.

In London, I thank: Shanaz Begum for her dedication, patience and steadfast companionship during 300 interviews; Rita Platts for her always joyful assistance at a moment's notice; and Helen Tindale, Carmel King and Samina Khan for sharing their passion and skills during the afterschool clubs. I am also grateful to Drs Caroline Fertleman, David Wickens, Martin Widschwendter, and Allison Jones for making room for ABBY in their laboratories.

In Sylhet, I am grateful to: Prof Lynnette Leidy Sievert, Dr Taniya Sharmeen and Dr Kesson Magid, for first introducing me to fieldwork in Sylhet and for their continued support since that first visit; Dr Osul Chowdhury, for his generosity of time, resources, understanding and wisdom all making Sylhet MAG Osmani Medical College the perfect home while in the field; the employees of the Microbiology Department at SOMC for hosting me and the samples; my host family for filling me full with Bengali delicacies and hospitality; my research assistants from Shahjalal University, especially Lutfunnessa Tania and Nazrina Chowdhury Bristi for their commitment to ABBY with the utmost grace.

In Bethesda, I am grateful to Dr Robert Hoover for me instilling in me the importance of simplifying the most complex problems and always having his door open; Dr Hormuzd Katki for his dedication to being my mentor in every sense of the word (from statistics to sanity); Dr Rebecca Troisi, for her good-humoured, timely, and precise advice in fieldwork, analysis and writing; Dr Regina Ziegler, for her keen insight to all things hormone measurement and her genuine encouragement along the way; Dr Francesco Barone Adesi, for his amazing ability to explain any topic in epidemiology and STATA with just a knock on his door; and Dr Sarah Daugherty for her compassionate insight into navigating a PhD.

Finally, at home, I express my gratitude to Stacy Antoville for designing the ABBY logo and for being the most encouraging friend and LeeAnn Marhevsky for her final editorial review. I am grateful to my parents for their limitless love and support, especially to my mother who knew that colourful bracelets would recruit 500 girls, and to my father for his astute review of each chapter always with a bit of humour. Lastly, I am indebted to my husband, Gregory Silverman, because beyond cooking with children, handling specimens and inputting data he has selflessly nourished my body and soul in every village this PhD journey has taken us.

PREFACE

Since the 1980's biological anthropologists set out to compare reproductive function among women. Peter Ellison and his colleagues' research broke new ground in the area of reproductive ecology by documenting great variation in ovarian function among women in Poland (Jasienska and Ellison 2004), Bolivia (Vitzthum et al. 2002), Zaire (now Democratic Republic of Congo) (Bentley et al 1998) and Nepal (Panter-Brick et al. 1993). These studies suggest that low levels of reproductive hormones are not dysfunctional ovarian function but an adaptive response to ecological factors. Ellison (1996) hypothesized that the adaptation of reproductive function to ecological contexts begins during childhood development.

Professor Gillian Bentley and colleagues tested the effects of developmental ecology on adult reproductive hormones in a series of Bangladeshi migrant studies (Núñez-de la Mora et al. 2006, Magid 2011, Begum 2011). They collectively found that adult reproductive hormones measured in individuals who migrated as children were more similar to hormones measured in second-generation and white British women, than those measured in individuals who migrated as adults or who had never migrated from Bangladesh. The collective findings from these studies suggest that there is a critical window during development that affects adult reproductive function. The juvenile period may be this window.

Yet, what is this unique stage in the life course called juvenility? This thesis aims to answer this question from a biocultural perspective. I designed the ABBY Project to explore age differences, hormonal variation and lifestyle factors associated with the juvenile period. It is a migrant study that collected individual-level data from girls and information on the wider cultural context to address whether juvenility varies by ethnicity, ecology and migration. To answer this question, ABBY's research objective is: to assess whether there is variation (biological and cultural) in the timing and experience of juvenility across populations.

In six chapters, I review the theoretical background of human development from evolutionary, cultural and psychological perspectives (Chapter 1), describe methods (Chapter 2), present the findings in three results chapters (Chapters 3-5) and discuss the findings (Chapter 6). Chapter 1 reviews literature pertaining to child development from across disciplines and sets up the exploration of juvenility within a migrant study. The overarching hypothesis is that juvenility will differ among populations and the patterns of juvenile development will differ according to ecological factors experienced during childhood.

Hypotheses based upon the life-history model are tested according to methodology detailed in Chapter 2 and presented in three results Chapters. Chapters 3 and 4 explore the endpoints of the juvenile period as defined by biological markers. Chapter 5 considers growing up in the context of migration and explores juvenility using cultural markers. The structure of this thesis deviates somewhat from traditional theses, in that the three results chapters have been designed as stand-alone papers each with independent background, methods, results and discussion sections. Yet, to avoid redundancy, many methods have been cross-referenced with Chapter 2 within each results chapter, and some additional literature review is included in the results chapters.

Chapter 3 focuses on adrenarche: the transition from childhood into juvenility. I test whether age and size at adrenarche differ by ethnicity, ecology and migration status. The timing of adrenarche, as defined by DHEAS, is compared across groups of girls according to migration group and body size. Adrenarche is measured by both hormonal and somatic markers and the timing of these markers are compared to each other. The specific predictions are that white British and British-Bangladeshi girls who spent all of their lives in London will reach adrenarche at an earlier age, report more somatic signs of adrenarche at an earlier age, be taller and fatter, and show higher DHEAS levels than Bangladeshi girls who live in Sylhet. Girls who spent part of their lives in Bangladesh before moving to the UK will reach

adrenarche earliest, report somatic signs of adrenarche earliest, be tallest and fattest, and show the highest levels of DHEAS.

Chapter 4 investigates puberty: the transition from juvenility to adolescence. I begin by testing whether age and size at pubertal onset and maturation differ by ethnicity, ecology and migration. Pubertal onset is measured by thelarche and pubarche, while pubertal maturation is measured by menarche. The timing of these events is compared across migration groups and by anthropometric quartiles. Urinary oestrogen levels are also compared across migration groups and pubertal stages. The predictions are that white British and British-Bangladeshi girls who spend all of their lives in London will reach thelarche, pubarche and menarche earlier, be taller and fatter at these times and will show higher oestrogen levels than Bangladeshi girls who live in Sylhet. Girls who spent part of their lives in Bangladesh before moving to the UK will reach thelarche, pubarche and menarche earliest; be tallest and fattest, at these times and will show the highest levels of oestrogen levels.

The second part of Chapter 4 compares the interval between adrenarche and pubertal stages across migration groups. If girls have an earlier adrenarche, are they also more likely to reach puberty early? The prediction is that the order and tempo of juvenile development will be similar across all migration groups due to similar phylogenetic constraints present in all girls.

Chapter 5 explores the experience of growing up as a British-Bangladeshi girl in London. I ask whether acculturation is a unidirectional process for Bangladeshi migrant groups living in the UK and whether the psychosocial experience of growing up runs parallel with biological development.

Chapter 6 draws conclusions from the findings of the project as a whole at both the proximate and ultimate levels of inquiry. The findings from ABBY extend to the field of developmental

origins of health and disease, where early life trade-offs between competing physiological requirements predict adult health outcomes later on in the life cycle.

CHAPTER 1: INTRODUCTION AND BACKGROUND

SUMMARY

The ABBY (Adolescence among Bangladeshi and British Youth) Project explores the relationship between migration and growing up from a biocultural perspective. This project tests for evidence of facultative adaptation to different developmental environments in the human female during the juvenile transition from child to adolescent, based on evolutionary hypotheses. The hypotheses are tested using a migrant study encompassing contrasting conditions within three variables: ethnicity, ecology, and migration. The first contrast is between two ethnic groups, Bangladeshis and white British. The second contrast is between the ecologies of Sylhet, Bangladesh and London, UK. The third contrast is among Sylheti, British-Bangladeshi migrant groups and white British girls. I explore these explanatory variables through comparisons of both biological and cultural markers of development among a sample of 488 girls, aged 5 – 16 years.

In this Chapter, I begin by contextualising the outcome variable - juvenility - within an evolutionary framework of life cycles. I then synthesize biological, cultural and psychological models of human development in order to position the juvenile period within the human life course. Next, I break the juvenile period into its component endpoints - adrenarche and puberty - which are the component outcome variables for this study. I review the current understanding of these stages and describe how they are assessed through hormonal, somatic, and cultural markers. I discuss if and how the timing of adrenarche and puberty vary by ethnicity and ecology and make the case for testing variation in juvenile timing within a migrant context. Finally, I propose hypotheses based on life history theory to test for predicted differences in juvenile development within a migrant study and pose questions regarding how acculturation interacts with migrant status in relation to growing up.

JUVENILITY, EVOLUTION AND THE HUMAN LIFE COURSE

It is important to view organisms as life cycles rather than with life cycles because it is the life cycle, not just the adult, which evolves (Bonner 1965). The life cycle is a strategy to answer such questions as: how fast to grow, when to mature, how much to reproduce and how long to live, in a collective effort of optimal reproductive success (Stearns 1992; Konner 2010). The human life cycle stands in stark contrast to other primates due to an extended period of dependency and delayed age of sexual maturity. This prolonged stage in human ontogeny evolved as "a mechanism that allows for more precise 'tracking' of ecological conditions via developmental plasticity during the growing years" (Bogin 2001: 120). Natural selection operates before reproductive maturation and during development, which is a particularly critical part of the life cycle. Moreover, there are periods during development that are particularly sensitive to ecological factors. The juvenile period is probably one of the most plastic times of development in the human life course (Hochberg 2008).

Humans grow more slowly and reach sexual maturity later than other primates, creating a prolonged period of development that includes childhood, juvenility and adolescence. In relation to the human life course, there is wide variation in patterns of growth and age at sexual maturity both within and between populations. Many studies have documented the variation in child growth and adolescent maturity, however less is known about whether there is variation in the timing of the juvenile period across human populations.

Disciplines position juvenility within the human life course in different ways. Within the evolutionary framework- which is taken to be the point of reference for this study- the juvenile period is a unique stage, inserted between childhood and adolescence. Juvenility occurs in girls between the ages of ages 7 – 10 years. Juvenility is characterized by a slow growth period (Tanner 1978) and a cognitive transition to increased capacity for learning

social skills (Campbell 2006). Juvenility also implies two transitional periods that are also only experienced by humans: at one end the transition from childhood to juvenility and, at the other, from juvenility to adolescence (Hochberg 2008). The former transition is marked by adrenarche and the latter by puberty.

Other frameworks of human development do not distinguish between juvenility and childhood, but do distinguish between early and middle childhood. The developmental frameworks included in Figure 1 all associate increased cognitive ability and social interaction with juvenility or middle childhood in some way. In psychology, middle childhood marks increased cognitive ability and the emergence of the sense of self, often referred as the 5 – 7 shift (Nelson 1993; White 1996). In psychosocial theory, Erikson's "Stage of Latency" includes the capability of learning and increased social interaction (Erikson 1968). Similarly, the Bengali stage of Balyakat (school age) is when partial reasoning begins, as well as the cultural learning of sex and gender roles (Aziz and Maloney 1985).

These frameworks of development suggest an obligate trajectory that associates developmental stages with characteristics during certain ages. Alternatively, it is worth viewing human development from a sociological perspective that acknowledges other dimensions of childhood, specifically how children locate themselves and are located in the social world (James et al. 1998). These authors suggest that children begin socialisation very early in life and that understanding socialisation may be limited if one unwaveringly associates the process with particular ages. Despite differences across models and perspectives, there is an overall recognition of a distinct stage in development marked by both biological and social abilities (highlighted in yellow in Figure 1). For this thesis' purpose, I use the terms juvenility and the juvenile period interchangeably to refer to this unique stage in the human life course.

Figure 1: Theoretical Frameworks of Development and the Life Course

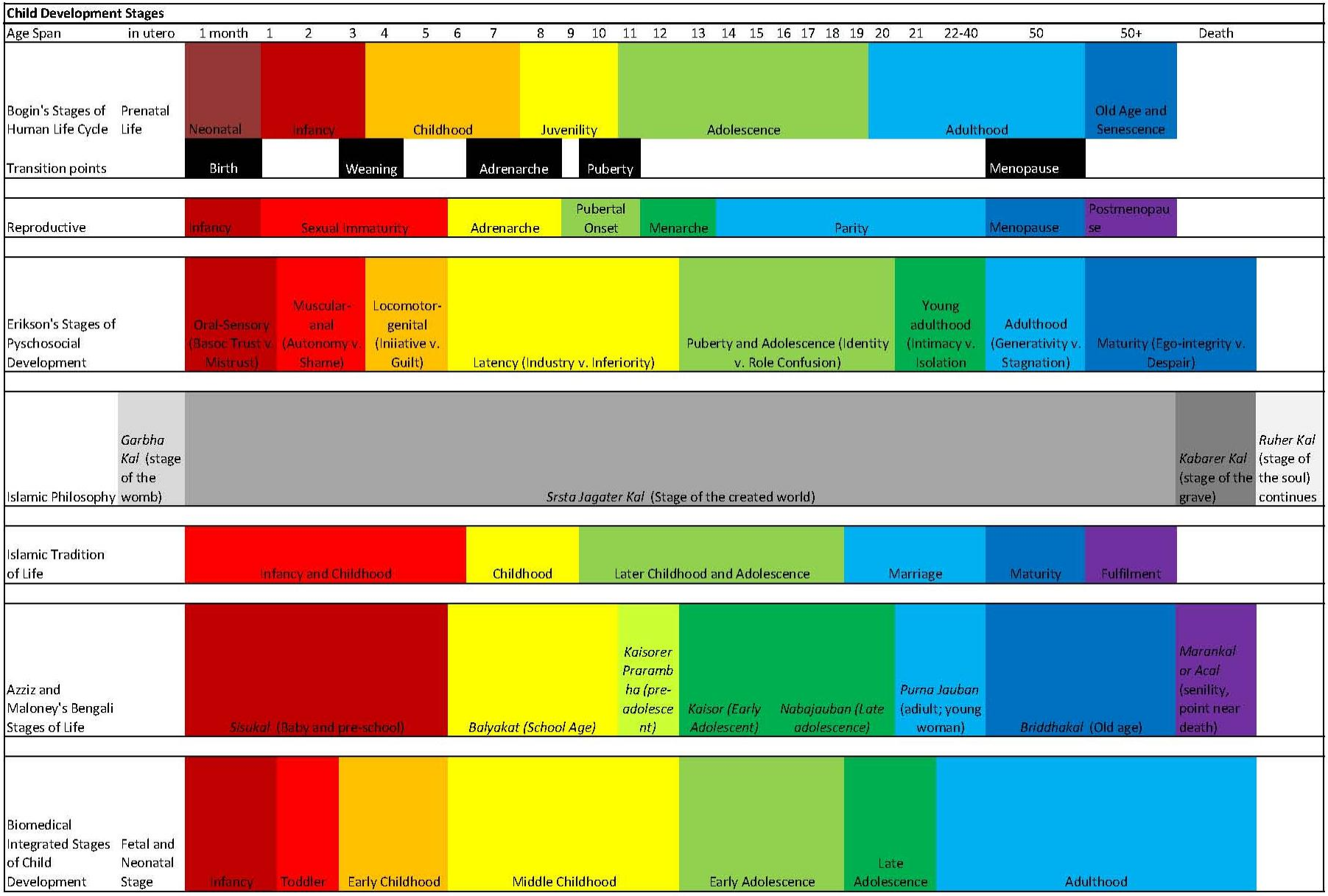
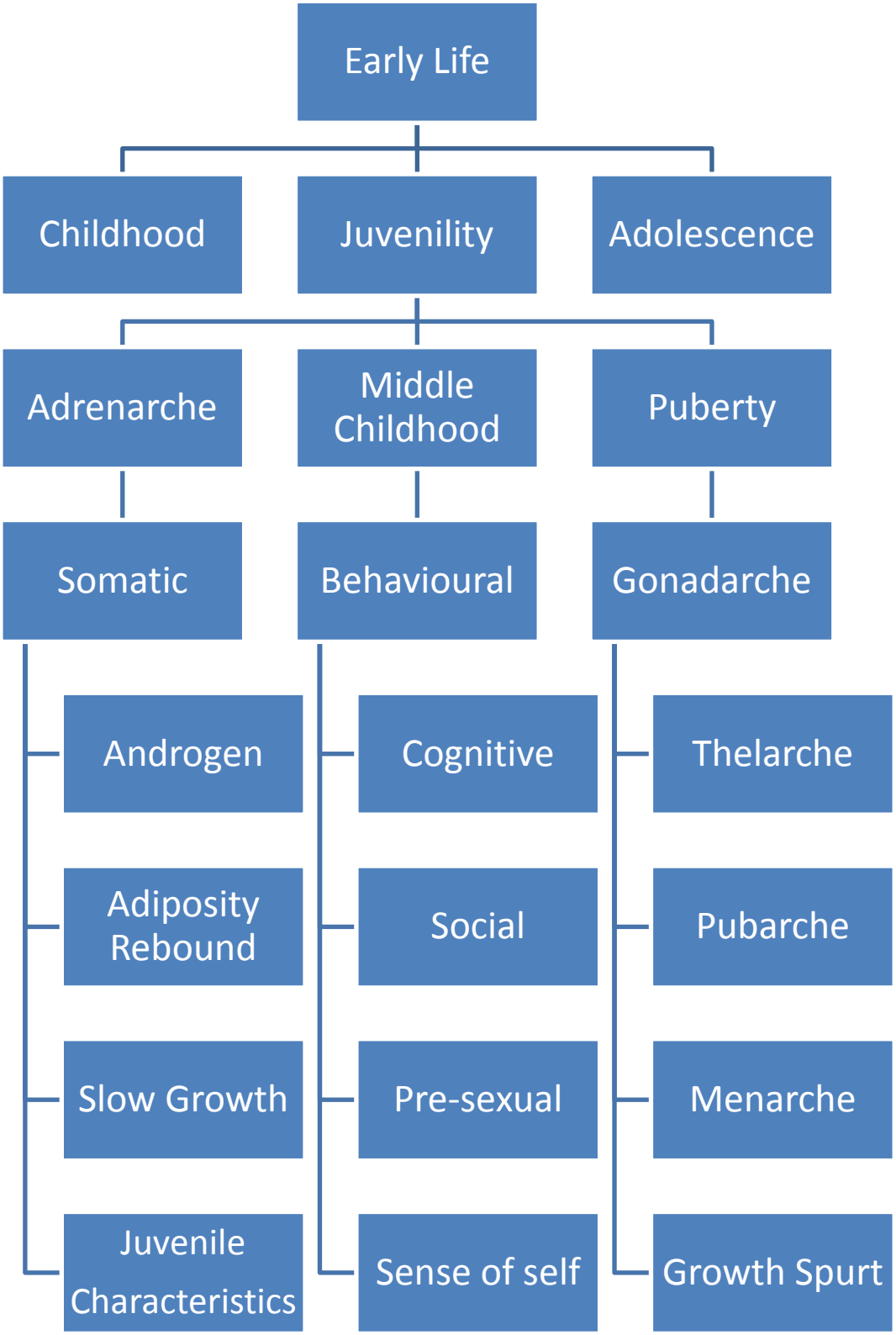


Figure 2: Evolutionary Model of Juvenility in the Context of Early Life



Overall, juvenility can be characterised by somatic and behavioural changes (Figure 2). The somatic changes include a decline in growth rate, an adiposity rebound (the pre-adolescent rise in body mass index (BMI)) (Rolland-Cachera et al. 1984; Rolland-Cachera et al. 2006) and the appearance of juvenile secondary sex characteristics including sexual hair and sebaceous gland development. These latter changes are said to be the physical manifestations of adrenal androgen production, which is marked by adrenarche. The behavioural changes not only include the aforementioned increases in cognitive and social abilities, but also the increased independence in self-feeding (Bogin 2001) and acquaintance with cultural practice. As Lancy and colleagues (2011) suggest, juvenility begins a period of "making sense" of the social world.

THEORIES OF IDENTITY DEVELOPMENT

Growing up from child to adult entails social maturation and developing a self-identity is part of this process. Different theoretical models of identity formation situate identity emerging during a critical incident, which can be characterized as either a time of crisis (Erikson 1968), exploration (Phinney 1989) or encounter (Cross 1991; Cross Jr 1995). Erik Erikson's psychosocial model centres on the identity of the individual, or ego. Erikson (1968) characterises the fifth stage of his eight stages of the human life cycle as developing an identity versus identity confusion; and, he marks adolescence as the crucial time when an individual searches for and develops one's identity. Alternatively, social identity theory does not focus on developmental stages of childhood per se, but centres identity formation around one's interaction with a larger social group-- how one identifies with that group and how the larger social context values that group. Tajfel's social identity theory differentiates between

those individuals who belong to highly valued groups that do not need to focus on their social identity and others who belong to devalued groups that may have to engage in a process to negotiate the meaning of their identity (Tajfel and Turner 1986). Being a part of a devalued group presents a time of crisis (ibid). Like social identity theory, racial identity theory (Cross 1991, 1995) also points to perceived discrimination as an encounter that stimulates reflection about the meaning of one's ethnicity. Therefore, unlike Erikson's model which implies that identity formation is part of development, the latter two theories acknowledge that one's ethnicity plays a crucial role in identity formation.

Adolescence and Ethnic Identity

Within a developmental trajectory framework, identity becomes salient during adolescence. Phinney (1989) extends Erikson's model to ethnic identity and introduces a period of exploration after unexamined ethnic identity and before reaching a committed ethnic identity. After a peak during middle adolescence, ethnic identity exploration is expected to decrease, as older adolescents become more secure in their personal and social identities. Pahl and Way (2006) measured Phinney's second stage, ethnic identity exploration, in a longitudinal study and characterised adolescence by decelerating levels of exploration after age 16. Ethnicity and perceived discrimination by peers moderated this pattern while immigrant status and gender did not. French (2006) compares identity exploration between early (age 11) and middle adolescence (age 14) and found that middle adolescents were more likely to increase in exploration than were early adolescents, suggesting that ethnic identity exploration is more likely to be stimulated during the middle adolescent years. While few studies have examined ethnic identity in children under the age of 10, some studies point to even earlier development of ethnic identity. Bernal et al. (1990) demonstrated the acquisition of ethnic identity

accompanying cognitive ability among nursery and primary school-age children. These researchers define ethnic identity more simply than Phinney and Cross do; they define ethnic identity in this age group as knowledge about one's own ethnic group and the sense of self as a member of that group. Perhaps coincidentally, adrenarche, argued to mark the emergence of the sense of self, also occurs during the transition from nursery to primary school.

LIFE HISTORY THEORY APPLIED TO JUVENILITY

Life history theory seeks to explain the variation in the life cycle by the concept of trade-offs. Life cycles differ by life history traits, such as age and size of maturity, which are bound together by trade-offs, such as survival versus current reproduction and current versus future reproduction (Stearns 1992). The function of trade-offs is central to life history theory and is used to explain evolutionary adaptation and short term development. Across human populations, these trade-offs shed light on how humans are able to adapt differently within varying environments and how much of an influence external factors have on the growth and development of the human body. Plasticity, the ability of a single genotype to produce many phenotypes during development, enables these trade-offs (West-Eberhard 1989). Life history theory also relates these trade-offs to ecologies.

Ecology determines the amount of resources available to an organism. When there are limited resources, the body must allocate energy to maintenance, growth or reproduction. As a result there are physiological trade-offs between these competing bodily functions which affect the life history traits, which in turn affect the life cycle (Stearns 1992; Charnov 1993).

While there is a phylogenetic constraint on how much plasticity is possible (Maynard Smith et al. 1985), life cycles can adjust either through genetic selection or through facultative

adaptations. Facultative adaptations are sensitive to environmental factors, are compromises between genetically fixed or malleable solutions, and result in plasticity. When human development is separated it into stages such as childhood, juvenility and adolescence, some life history traits, such as adrenarche and puberty, may prove to be more plastic than others.

The variation in child growth and timing of maturation in response to improved nutrition and decreased infectious disease can be viewed either as a facultative adaptation or physiological trade-offs during development (Worthman 1999). Some trade-offs are genetically controlled (Roff 2007) so that competing individuals differ in their (fixed) relative distribution of energy to growth versus reproduction. Thus, populations in ecologically stressed environments may have different life cycles than populations in ecological abundant ones. For example, if a child grows up in an environment with poor nutrition and is exposed to infection, the body will invest its energy into maintaining basic physiological functions rather than growth or reproduction. These trade-offs appear as small size and late age at maturity. However, the timing of maturity may also be the result of psychosocial rather than solely energetic stress.

An important framework for analysing psychosocial influences on pubertal processes is Belsky et al.'s (1991) evolutionary theory of reproductive development, which focuses on the role of familial and ecological stressors in accelerating pubertal maturation. Drawing on life history theory, this theory posits that "a principal evolutionary function of early experience—the first 5 – 7 years of life—is to induce in the child an understanding of the availability and predictability of resources (broadly defined) in the environment, of the trustworthiness of others, and of the enduringness of close interpersonal relationships, all of which will affect how the developing person apportions reproductive effort" (Belsky et al. 1991: 650). An implication of this differential susceptibility, as articulated by Belsky (2000), is that the small main effects of parenting processes on pubertal timing may overestimate the impact of family environments in some children (low susceptibility, more fixed reproductive development)

and underestimate it in others (high susceptibility, more plastic reproductive development). Neither the developmental adaptive response theory nor the psychosocial acceleration theory has been applied to the juvenile period and tested in a migrant study of girls as I intend to do in this thesis.

THE JUVENILE PERIOD, ETHNICITY AND ECOLOGY

The juvenile period begins with adrenarche and ends with the onset of puberty. In the following section, I will define each of these endpoints, summarise how they are measured and review if and how the timing of each vary by ethnicity and ecology.

ADRENARCHE

Adrenarche marks the transition to juvenility (Hochberg 2008). Adrenarche is the production of adrenal androgens during middle childhood and typically occurs between the ages 6 – 8. The rise in adrenal 19-carbon (C19) steroids, primarily dehydroepiandrosterone (DHEA) and its sulphated form dehydroepiandrosterone-sulfate (DHEAS), corresponds with the widening of the innermost layer of the adrenal gland, the zona reticularis (Dhom 1973). The physician Fuller Albright termed this entrance of adrenal androgens as adrenarche (1947). DHEAS, the most abundant androgen in circulation, is present in *utero*, declines soon after birth and rises again around the ages of 6 – 8 when adrenarche occurs (Reiter et al. 1977). DHEAS levels then continue to increase steadily into the second and third decade of life and remain relatively constant until they decline with senescence, which Albright also named adrenopause (Albright 1947). While androgens steadily rise through most of early life, the first peak in DHEAS marks adrenarche. I now turn to different disciplines to explore why

adrenarche evolved in humans and is hypothesized to play a role in the extension of the pre-adolescent phase of human ontogeny (Bernstein et al. 2012).

In terms of comparative biology, adrenarche was thought to only occur in humans and great apes, although the current view in comparative biology suggests that the definition of adrenarche needs to include morphological, biochemical, and endocrine evidence of zona reticularis development so that adrenarche can be better compared across species (Conley et al. 2012). Conley et al (2012) have recently suggested that adrenarche also occurs in infant rhesus macaques, albeit earlier and during a narrower developmental window than humans and some great apes. There is still debate, however, about when exactly adrenarche appeared in the evolutionary history of hominidae (Hochberg 2008; Bernstein et al. 2012), but it is suggested to have evolved to promote brain development (Campbell 2006; Campbell 2011a). For example, DHEAS may protect brain plasticity by promoting prolonged development of the human prefrontal cortex (Chugani 1998; Campbell 2011).

Developmental psychology has paid the most attention to this transitional time, coining it the 5 – 7 shift (Piaget 1963). The 5 – 7 shift occurs after the completion of brain growth in weight (Cabana et al. 1993) and has been found in all cultures in which it has been investigated (Rogoff et al. 1975). During the 5 to 7 shift, a child's cognitive ability, self-memory (Nelson 1993) and theory of mind, or metacognition (Konner 2010) are established, which collectively lead to greater interaction with peers and adults (Campbell 2006). When participating in psychological games, children across many cultures universally display more self-awareness and the ability to process information after age 5 (Konner 2010). Therefore, the period after the 5 – 7 shift and before puberty is speculated to have evolved to allow more time for brain development, subsequent acquisition of technical skills, and the development of complex social roles and cultural behaviour.

In terms of culture, adrenarche may begin an essential period for social learning. Lancy and colleagues (Lancy and Grove 2011) looked at the ethnographic record and compared markers of middle childhood across cultures. They observed a universal recognition of a shift in behaviour during middle childhood that in turn gets noticed by adults, albeit less ceremonially than the arrival of puberty. Also, during adrenarche the social interactions of a child extend from the family to the neighbourhood context and this transition increases opportunity to learn cultural rules and behaviours. For example, during middle childhood children take on and are seen as capable to be given social responsibilities such as caring for siblings, carrying wood and food production (Weisner 1987; Blurton Jones 1993). In western settings, most children start formal school between the ages of 5 and 6 and this may reflect cultural recognition that children are now more capable of learning and participating in society. At age six, there is also a marked difference in children's understanding of family roles (Watson and Amgott-Kwan 1983). While at age 3 a child can identify characteristics of a mother, at age 6 she can also understand that a daughter can become a mother and that a woman can simultaneously be a daughter, mother and grandmother (Konner 2010). This understanding of familial roles occurs at the same time that children start to become sibling carers. Konner (2010) proposes that sibling care is one adaptive function of middle childhood. The extended period of cultural learning during middle childhood equips humans with the social skills necessary for future social interactions and reproduction, which ultimately, in addition to other environmental clues, help to determine evolutionary fitness.

In relation to sexual behaviour, changes in behaviour during middle childhood can be argued as gender-specific and pre-sexual. During middle childhood, same sex friendships are secured as boys and girls choose to play with their own gender. Among hunter-gatherers, where sex taboos are relaxed, children start to engage in play sex around this age (Frayser 1994), but Konner (2010) compares play sex to play fighting, highlighting that the emotions and

behaviours of playing are very different from the real thing. Furthermore, heterosexual attraction first occurs at 10 to 10.5 years for girls, so the relationships occurring after adrenarche but before adolescence are arguably pre-sexual (Herdt and McClintock 2000). The middle childhood period becomes a safe period or cushion for social skills to develop before the onset of gonad-driven hormones and sexuality. In other words, adrenarche marks a stage designated for the brain and social body to mature before the reproductive body matures. In fact, Ben Campbell (2006) refers to adrenarche as "prepubescent adolescence". If and how adrenarche is associated with pubertal maturation is explored further in Chapter 4.

Markers of Adrenarche

Adrenarche is marked by changes in adrenal androgen production. The substantial rise in DHEAS between 6 – 8 years of age was first documented by Reiter et al. in 1977 by measuring serum DHEAS among healthy boys and girls (Reiter et al. 1977). Subsequent studies have also observed a rise in DHEAS and have established that once DHEAS levels reach 40-50 µg/dl (1.1 nmol/l), a child is said to cross the threshold into adrenarche (Reiter et al. 1977;Wierman et al. 1986Havelock et al. 2004). However, there is some suggestion that adrenarche may occur earlier if markers other than DHEAS are utilised. Adrenal androgen production is associated with temporal and spatial morphologic changes in the zona reticularis, the inner most layer of the adrenal gland (Dhom 1973, Parker et al. 1983, Suzuki et al. 2000, Rainey et al. 2002). A cross-sectional study of adrenal gland biopsy showed that the zona reticularis developed as early as 3 and as late as 10 (Dhom 1973), while another study of young adrenal autopsy specimens documents the expansion of the zona reticularis after 4 years of age (Hui et al. 2009). Both studies found that a continuous layer of 'functional' zona reticularis appears at age 6. More recent studies measuring the sum of urinary C19 androgens, such as androsterone and etiocholanolone, observed a consistent rise in androgens as early as age 3 years (Remer et al. 2005). Auchus (2011) argue that thinking

of adrenarche as an "event" in middle childhood has clouded our thinking about the process of adrenarcheal development. However, Remer et al.'s (2005) study demonstrated that DHEAS levels are first detectable in circulation at age 7 years, meaning that the DHEAS definition of adrenarche still holds. Therefore, biologically, adrenarche most likely is a gradual process that begins in childhood, lasts until young adulthood and encompasses the gradual rise in adrenal androgen production (Remer et al. 2005; Auchus 2011). For research purposes, however, adrenarche is defined by serum DHEAS levels above 40 µg/dl (Reiter et al. 1977,Wierman et al. 1986, Kurtis et al. 2001, Havelock et al. 2004).

Adrenarche can also be marked by the physical characteristics that reflect androgen production including axillary hair, oily skin and body odour (Kaplowitz et al. 1986, Auchus and Rainey 2004, Campbell 2006). The skin is not capable of direct synthesis of androgens, but it contains all the enzymes necessary to convert the pro-hormones DHEA and androstenedione into more potent androgens such as testosterone and dihydrotestosterone (DHT) (Zouboulis 2009). Pilosebaceous units, the androgen-sensitive components of skin including hair follicles, sebaceous glands and sweat glands, each metabolize androgens in a characteristic pattern (Rosenfield 1986) resulting in physical characteristics. In response to increasing levels of androgens, pilosebaceous units become large terminal hair follicles in sexual hair areas. Androgens promote sexual hair growth by recruiting a population of pilosebaceous units to switch from producing vellus hairs to initiating terminal hair growth. In sebaceous areas, androgens promote sebaceous gland activity resulting in oily secretions called sebum from the skin (Stewart et al. 1992). In addition to sebaceous glands, sweat glands account for the vast majority of androgen metabolism in skin. Peripherally converted adrenal androgens are responsible for the development of apocrine glands within the skin, resulting in body odour (Auchus and Rainey 2004). Thus, researchers hypothesize that body odour is also a reliable marker of adrenarche (Campbell 2011a).

Timing of Adrenarche

The timing of adrenarche may be relatively hardwired and fixed or plastic. A twin study suggests that 65% of the variation in adrenarche timing is genetically explained (Li and Ji 2007), and concludes that serum DHEAS concentrations could be mainly influenced by genetic factors. No biological trigger for adrenarche is known, although some have been postulated. Adrenocorticotropic hormone (ACTH) and corticotropin-releasing hormone (CRH) have been proposed as a dual control mechanism (Archer and Chang 2004). However, both regulatory hormones probably do not modulate the production of adrenal androgen secretion. It has been postulated that there is another "ACTH-like" pituitary factor, which might stimulate adrenal androgen secretion or that the trigger comes from the adrenal gland itself (Grumbach 1980). Without knowing the trigger, it is unclear how the timing of adrenarche is regulated by the interaction of genetic and environmental factors.

Ethnic and Ecological Variation in Adrenarche

There is some, but not extensive, evidence for variation in the timing of adrenarche according to ethnicity and environment. According to Pratt et al. (1990), African American children between 5 and 10 years of age excreted 17% higher levels of DHEAS than white children of the same ages (Pratt et al. 1990). In a natural experiment with Peruvian children living either at sea level or at high altitude, age at adrenarche occurred later among girls living at high altitude (Goñez et al. 1993). However, in a study of adolescents aged 9 – 17 years in a later-maturing central highlands Kenya population of Kikuyu agriculturalists, levels of adrenal androgens rose before age 9, suggesting adrenarche occurred at the same age as in western girls (Worthman 1986). With so few comparisons among healthy children across ecological contexts, it is unclear whether the timing of adrenarche varies cross-culturally, but studies within populations suggest that specific environmental factors influence the timing of adrenarche.

PHYSIOLOGICAL AND PSYCHOSOCIAL MEDIATORS OF ADRENARCHE

Low birth weight, body fatness, psychosocial stress and immune function have been associated with variation in adrenarcheal age or, more specifically, an exaggerated adrenarche, indicated by elevated levels of androgens at early ages. Being relatively small at birth as a result of prematurity and intrauterine growth retardation is associated with premature adrenarche (Auchus and Rainey 2004). Spanish girls, aged 11 – 19 years and post-menarche, who were born small for gestational age (SGA) had DHEAS concentrations two-fold higher than girls born at normal weight for gestational age (Ibanez et al. 2011). SGA is linked with polycystic ovarian syndrome (PCOS) and, among daughters of women with PCOS, 12.5% of girls in childhood and 32.4% in peri-puberty presented biochemical evidence of an exaggerated adrenarche (Maliqueo et al. 2009). However, another study among Finnish girls found that premature adrenarche was not associated with small birth size (Utriainen et al. 2009).

A foetal programming component, as evident among SGA babies, may influence the timing of adrenarche, but a second cue during middle childhood may be necessary to activate the process. Adrenal androgen concentrations are highest in those small infants who become heavier than average during early childhood. Ong and colleagues (2004) found that lower birth weight and higher current weight was associated with higher DHEAS among UK children. There is also evidence for a causal relationship between nutritional status and adrenarche based on Remer et al.'s (2000) longitudinal study that demonstrated increased DHEAS levels during the times of increased BMI, independent of chronological age or Tanner Stage. Obesity and leptin, a marker of adiposity, are both associated with increased adrenal androgens among pre-pubertal children (Genazzani et al. 1978), supporting the relationship that body fat is associated with the onset of adrenarche.

Psychosocial stress may adjust the timing of adrenarche as well. Higher quality parental investment and less familial conflict forecast a later adrenarche (Ellis and Essex 2007). Girls with premature adrenarche have significantly more oppositional defiant disorder and higher symptom counts reflecting anxiety, mood or disruptive behaviour disorders. This suggests that such girls may be more vulnerable to psychopathology than girls who reach adrenarche after age 6 (Dorn et al. 2008). Ellis and Essex (2007) also suggest that the stress response system may be up-regulated by acutely stressful or exceptionally supportive psychosocial environments during juvenile development.

The timing of adrenarche timing may be affected if the immune system is challenged. Adrenal production of DHEA and DHEAS appears to be modulated by elements of the immune system. Levels of adrenal androgens differ depending on whether an immune challenge elicits a pro- or anti-inflammatory response. DHEA and DHEAS may initially be stimulated in circumstances of trauma, infections and other sources of physiological stress.(Chen and Parker 2004). However, these androgens are lower in adults with chronic immune and inflammatory states such as rheumatoid arthritis, human immunodeficiency virus, and autoimmune deficiency syndrome (ibid). Even in patients with rheumatoid arthritis, situations of acute stress, such as sepsis, activate the HPA axis, resulting in an acute rise of ACTH followed by increase in DHEA as well as cortisol (Cutolo et al. 1999). DHEAS has also been shown to reduce susceptibility to viral, bacterial and protozoan infections (Chen and Parker 2004). Despite not knowing how, it is apparent that the immune system and the production of adrenal androgens, including DHEAS, are related.

In summary, a number of ecological factors may alter the timing of adrenarche by acting as stressors along the HPA axis. Stress can generally be defined as the response of an organism to its environment (natural or social) in ways that prepare it to deal with the immediate allocation of energy and perceived or real threats to the organism's survival and reproduction

(Wiley 2009). Therefore, energetic (nutritional or immunological) or psychosocial (family context or migrant status) stressors may act systemically or locally along the HPA axis to adjust the timing of adrenarche either by increasing or decreasing androgen levels. If these stressors accelerate the timing of adrenarche, it is unknown what implications this has for the overall timing of juvenile development and overall health.

PUBERTY

Puberty marks the end of the juvenile period and the beginning of adolescence. The pubertal transition is a key period in the life course encompassing the morphological and physiological changes that occur as the human body transforms from a sexually immature to a mature form (Marshall and Tanner 1986). Puberty is not an isolated event, but rather a developmental continuum, the initiation of which stems from the central nervous system. In fact, sex steroids are present soon after birth, but then decline to non-detectable levels leading some to suggest that there is a "gonadastat" during childhood that suppresses sexual maturation until puberty (Weirman and Crowley 1986).

Puberty, as defined by endocrinology, is the re-initiation of the hypothalamic-pituitary-gonadal (HPG) axis, which is also referred to as gonadarche (Bogin 2001). In girls, gonadarche operates along the hypothalamic-pituitary-ovary (HPO) axis when the pulsatile production of gonadotropin-releasing hormone (GnRH) from the hypothalamus results in the pituitary producing more follicle-stimulating hormone (FSH). This signals the ovaries to start producing steroids, primarily oestrogens. Subsequently, oestrogens rise and cause the feminizing pubertal changes of girls such as breast development. Androgens also increase during puberty and manifest physically as sexual hair growth (Weisfeld 1999; Tanner 1978). Later in puberty, when oestrogens reach adult levels, the HPO axis can either operate as a

negative or positive feedback system, the latter of which activates ovulation (Weisfeld 1999). The switching between these two feedback systems results in menstrual cycling characteristic of an adult woman. While some argue that puberty is strictly gonadarche (Kaplowitz 2011), others suggest that puberty is composed of at least three distinct biological processes including adrenal maturation (adrenarche), gonadal maturation (gonadarche), and somatic growth (Wierman and Crowley 1986; Palmert et al. 2001; Maskarinec et al. 2005; Shirtcliff, et al. 2009).

MARKERS OF PUBERTY

The physical changes that take place during puberty can be divided into specific events. Puberty includes the following stages but not necessarily in this order: thelarche (breast development), pubarche (development of pubic hair), and menarche (first menstruation). Puberty is usually characterised by a period of fast growth with peak height velocity marking pubertal onset (Bogin 2001; Shi et al. 2010). Table 1 describes the definition, general age at onset, hormonal markers and secondary sexual characteristics associated with each stage of sexual development.

Thelarche, pubarche and menarche collectively reflect the overall increased production of sex steroids. Thelarche and pubarche reflect oestrogen and androgen production respectively, and the foremost is generally considered to mark pubertal onset (Kaplowitz 2006). Menarche marks the endpoint of the pubertal continuum but it may take two or three more years before regular ovulatory menstrual cycles begin. Thus, age at sexual maturity can be deemed after this sub-fecund period or by age at first birth.

Even though menarche is a memorable event for most women, determining the age at which this occurred retrospectively is not straightforward and there are methodological limitations

to consider when comparing menarcheal age across studies. There is more chance for recall bias among older women due to the time lapse since the event occurred.

Alternatively, the status quo method samples girls at different ages and asks whether they have started their period or not (Marshall and Tanner 1986). Statistical analyses, such as probit or survival models can then determine the age at menarche from such cross-sectional data. Ideally, the best way to determine mean ages at menarche across populations is through longitudinal studies but this is not easily feasible, especially using a migrant model.

Pubertal development is marked by the appearance of secondary sex characteristics including breast and pubic hair development. The majority of work comparing pubertal development has been framed by the Tanner staging technique devised by James Tanner and colleagues, which describes five stages of puberty ranging from no development (Stage 1) to final adult development (Stage 5) (Marshall and Tanner 1969). By standardising the intensity of both breast and pubic hair development in combination with the presence or absence of menarche, resulting normative ages associated with each Tanner Stage have been devised and, in short, the Tanner Stages have become the gold standard for measuring pubertal development. The age at pubertal onset is usually defined by when an individual reaches either breast or pubic hair Stage 2.

Table 1: Stages of sexual development as defined by biology, hormones and secondary sex characteristics

Developmental Stage	Age	Definition	Hormones	Secondary Sex Characteristics
Adrenarche	6-8	Early part of sexual development Puberty of the adrenal gland Maturation of the hypothalamic-pituitary-adrenal axis	Increase in adrenal DHEA and DHEAS Associated with the broadening of the zona reticularis in the adrenal cortex	Axillary and pubic hair Oily skin Body odour
Pubarche	11-12	Appearance of pubic hair Manifestation of adrenarche	Androgens and perhaps oestrogens	Pubic hair
Thelarche	10-11	Appearance of breast tissue Manifestation of gonadarche	Increase in oestrogen results in breast development	Areolar and breast development
Menarche	12-13	First menses	HPO axis is activated with increased levels of oestradiol and progesterone	Menstrual bleeding
Puberty Gonadarche	10- 15	Maturation of the hypothalamic-pituitary-gonadal (HPG) system The second component of sexual development Activation of gonadal function	Gonadotropin-releasing hormone pulse generator is reactivated	Breast development, pubic hair growth, growth spurt and skeletal maturation

OESTROGENIC MARKERS OF PUBERTY

Patterns of oestrogen production, particularly circulating oestradiol, can be used as biomarkers of puberty in girls. It is clear that oestrogen levels are higher among girls who have reached menarche compared to girls who have not. Preece (1986) found that the most dramatic rise in oestradiol occurs between breast stages 2 and 3, suggesting that there is a dose-response relationship between breast development and oestrogen levels. For clinical purposes, reference levels of serum steroids have also been associated with each Tanner Stage (Soldin et al. 2003). However, these references are based on western populations and may not reflect variation across other human groups.

There is variation and fluctuation in hormonal values underlying the physical manifestations associated with puberty. Following menarche, typically there is a period of 2 to 3 years, known as adolescent subfecundity, when the hypothalamic-pituitary-ovarian axis is being primed for, but has not resulted in, regular monthly menstrual cycling (Ellison 2002). Vihko and Apter (1980) illustrated among a group of girls that only 14% of cycles were ovulatory in first year post-menarche, 50% in the three years following menarche, and 87% six years following menarche. Reproductive steroid levels also fluctuate markedly over the course of a menstrual cycle, displaying a biphasic pattern with low steroids during the follicular phase, rising and peaking at ovulation and declining back to low levels during the luteal phase. Considering that there is variation in oestrogen levels due to anovulatory cycles or menstrual cycle phase, it is difficult to associate levels of ovarian hormones with secondary sex characteristics without taking into account the occurrence of ovulation.

Patterns of oestrogen production in the years preceding puberty are not well characterized. Whether the earlier appearance of breast buds and pubic hair reflects circulating ovarian

steroids (central puberty) as opposed to adrenal or adipose steroid production (peripheral puberty) is unclear (Kaplowitz 2011). Radfar (1976) found differences in oestrogen levels between girls aged less than eight years of age and older than eight even though all were in breast Stage 1, indicating that oestrogens present before breast development may not be the result of the HPO axis . While the ovaries produce the majority of oestrogens in women of reproductive age, oestrogens that are present in pre-pubertal girls may reflect other sources of oestrogen production. Oestrogens can be either synthesized centrally and de novo by the ovaries or peripherally from the conversion of adrenal androgens in adipose tissue (Stanczyk 2009). Specifically, DHEAS is a precursor to oestrone and oestradiol which can be synthesised in adipose tissue via aromatase (ibid). It may be that oestrogens converted from androgens are present before puberty but not at adequate levels or in the necessary pulsatile fashion to stimulate the development of secondary sex characteristics. There is further debate about whether the early appearance of secondary sex characteristics reflects production of endogenous steroids or exposure to exogenous steroids in the environment. There is an imperfect match of hormones with physical measures of puberty due to variation in the source of hormones and the production of hormones over the course of a day, month and year. Nevertheless, knowledge of the underlying hormonal processes provides information about pubertal maturation not available from overt physical measures.

TIMING OF PUBERTY: CHRONOLOGY, ORDER AND TEMPO

The timing of pubertal development is relatively plastic and can be assessed by different measures including chronology (age), order (pathways), and tempo (duration/ intensity of each stage). It is important to separate out these measures when assessing if the timing of these events differ by ethnicity, ecology and migration.

Chronological Age

The chronological age at which puberty occurs is a relatively straightforward measure with which to compare development across populations. Age at menarche, a milestone event in a woman's life, is the most widely used marker of pubertal maturation. By comparing the average age at menarche across populations, one can see that the timing of menarche is influenced by the environment in addition to genetic background. Twin studies suggest that 57-82% of variation in the age at menarche can be explained by heritable factors (Dvornyk and Waqar-ul-Haq 2012). On the other hand, age at menarche also differs according to ecological context. Population medians of age at menarche vary from 12 years in urban post-industrial societies to 18 years in rural subsistence groups (Worthman 1999). Countries with favourable levels of nutritional and living standards tend to have populations who reach menarche at earlier ages than populations facing less favourable conditions. In the 1960s, Tanner documented the average age at menarche to be 13.5 years among English girls living in a children's home. In 1977, Chowdhury et al. reported the mean age at menarche as 15.9 years for Bangladeshi Muslim girls living in a rural area. Haq (1984) demonstrated that urban Bangladeshi girls reached menarche on average about 3 years before rural girls. Furthermore, among northern European countries there have been secular declines in the age at menarche as living standards generally improved (Eveleth and Tanner 1991; Worthman 1999). However, since the 1960's, the secular decline in age at menarche has stabilised to around age 12 years in western countries (Kaplowitz 2006). Age at menarche continues to shift earlier among populations undergoing economic development as documented in China (Huen et al. 1997) and Mexico (Malina et al. 2004). Overall, age at menarche differs between populations by ecology, within populations by SES, and over time, yet this event occurs rather late in the pubertal transition.

Age at thelarche and pubarche are helpful measures of pubertal onset. Marshall and Tanner (1969) were the first to look at pubertal onset in addition to menarche and found that 95% of girls reached puberty between the ages of 8.5 and 13 years, suggesting that signs of puberty before age 8 should be considered precocious (Kaplowitz 2006). There is an acceptable five year window within which puberty can begin, suggesting that the timing of thelarche and pubarche can differ according to ecological factors. Marshall and Tanner (1969) reported the mean age at thelarche and pubarche to be 11.15 and 11.69 years respectively, from the same sample of English girls living in a children's home during the 1960s for whom they derived a mean age at menarche. In the 1990s, Herman-Giddens (who initiated the first study of pubertal onset in the US) found an earlier onset for both thelarche (9.96 years) and pubarche (10.51 years) among white Americans. Girls with higher BMIs also had an earlier breast development compared to girls of normal weight (Herman-Giddens et al. 1997); however, these studies may have been confounded by clinicians mistaking fat tissue for breast tissue. Herman Giddens (1997) and one other US study (Wu et al. 2002) also found earlier breast and pubic hair development among African-American and Mexican- American girls when compared to white Americans (Herman-Giddens et al. 1997; Wu et al. 2002). More age references for each pubertal stage can be found in Table 2, which illustrates that there is wide variation in the age at pubertal onset (thelarche and pubarche) according to population and ethnicity. Whether the international variation can be attributed to genetics or environment is not clear, however, the standard model of pubertal development based on white European populations may not be a truly appropriate model.

Table 2: Age at Thelarche, Pubarche and Menarche across International Studies

Study	Country/ Ethnicity	Statistic	Thelarche	Pubarche	Menarche
Marshall and Tanner (1969)	England	mean	11.2	11.7	13.5
Herman-Giddens et al. (1997)	USA				
	White	mean	10.0	10.5	12.9
	Black	mean	8.9	8.8	12.2
Wu et al. (2002)	USA				
	Black	mean	9.5	9.5	12.1
	Mexican		9.8	10.3	12.2
	White		10.3	10.5	12.7
Mahachoklertwattana et al. (2002)	Bangkok	median	9.4	11.1	11.2
Juul et al (2006)	Denmark	mean	10.9	11.3	13.4
Mul et al. (2001)	Netherlands	median	10.7	X	13.2
Huen et al. (1997)	China	median	9.8	11.6	12.4
Garnier et al. (2005)	Senegal	median	12.6	X	15.9
Kashani et al. (2009)	Iran	median	10.1	10.8	12.7
Boyne et al. (2010)	Jamaica	median	8.8	9.9	12.0
Russo et al. (2012)	Italy	mean	9.8	10.1	12.5
Woronkowicz et al. (2012)	Poland	median	10.3	10.6	12.7

Order of Pubertal Events

Marshall and Tanner (1969) summarized the temporal order of pubertal stages as breast, pubic hair and then menarche, as observed in the Harpenden Children's home. However, they also recognized that the order in which girls progressed through these stages may differ because 1/3 of girls whom they studied reached pubarche before thelarche (Marshall and Tanner 1969). In a recent updated review of the literature, Kaplowitz (2011) outlines the order of puberty as follows: thelarche, growth spurt and menarche. He argues that pubarche is not related to puberty per se as the appearance of pubic hair is regulated by androgens (Kaplowitz 2011). Other studies acknowledge both androgens and pubic hair as part of puberty (Biro et al. 2003; Martin et al. 2004). Biro and colleagues (2003) suggest that there are different pathways through puberty. A girl may progress through puberty synchronously, meaning that the breast development and pubic hair growth occur simultaneously and develop steadily until the end of puberty when menarche and regular cycling begin. However, Biro et al. (2003) found that as many as 52% of children progressed through puberty asynchronously, with either pubic hair or breast development manifesting before the other. While Biro et al.'s pathway framework is helpful in comparing patterns of pubertal development, aspects of their model need to be clarified. This study recruited children beginning at age 9 years, so it is possible the onset of either thelarche or pubarche appeared before enrolment. The Biro model also places children who showed signs of pubic hair first as progressing through the adrenarche pathway, which I later discuss, is an erroneous conflation of adrenarche and pubarche.

Tempo of Pubertal Development

Pubertal tempo in relation to the timing of adrenarche has seldom been explored in the literature. Variation in the tempo of puberty is not a new idea as it was mentioned in the pioneering work of Tanner and colleagues (Marshall and Tanner 1969), but theirs and

subsequent studies have focused on the tempo of development between thelarche and menarche. Marshall and Tanner (1969) reported that the mean interval from thelarche to menarche was 2.3 years in the Harpenden study. More recent studies have documented an interval of 3.3 years between thelarche and menarche suggesting that as thelarche occurs earlier, there is also a slower tempo through pubertal development (Kaplowitz 2011). Girls could either be progressing through puberty at a slower, but steadier tempo than before, or pubertal development is more punctuated, meaning breast development occurs first and then a few years later menarche happens. Kaplowitz (2011) suggests that punctuated pubertal timing among contemporary girls may be a new variant of pubertal maturation compared to girls who progress through puberty at a more steady pace. Alternatively, earlier breast development may be the result of peripheral production of oestrogens in relation to increased adiposity and obesity (Jasik and Lustig 2008) or exposure to exogenous oestrogens in the environment rather than the manifestation of an activated HPO axis.

Increased pre-pubertal sex hormones may account for an earlier onset of puberty and faster pubertal tempo. Shi and colleagues (2010) report the first and only study to determine oestrogen excretion levels in relation to pre-pubertal development in healthy girls using growth rates in addition to Tanner staging. Their study found a relationship between pre-pubertal oestrogen levels and pubertal tempo in that increased urinary oestrogen levels were associated with a shorter duration of pubertal growth acceleration. Elevated oestrogens were also associated with a 0.9 year earlier onset of breast development and a 0.3 year earlier menarche respectively, when compared to girls with lower urinary oestrogen, even after adjusting for BMI. These authors did not find a relationship between oestrogen and pubic hair growth. However, Shi et al. (2009) attributed some of oestrogen's effects on breast development to local conversion from adrenal androgens during the pre-pubertal period.

Conversely, oestrogen's effect on predicting the timing of menarche was independent of adrenal androgens.

Pubertal tempo may also vary between early and late maturing girls. Apter and Vihko (1983) found pubertal development was faster in girls with an early menarche, as indicated by the significantly shorter time from both breast and pubic hair Stage 2 to menarche. These authors also looked at the tempo between reaching menarche and having ovulatory cycles and found a rapid onset of ovulatory cycles following an earlier menarche. The rate of progression through puberty to age at sexual maturity may be more informative for understanding early life effects on reproductive function than the actual age at which these stages occur. Even less is known about the tempo of development between adrenarche and puberty.

Recent studies suggest that the timing of adrenarche and levels of adrenal androgens may fine-tune pubertal timing. Higher levels of juvenile adrenal androgens may precipitate an earlier onset of breast and or pubic hair development. Remer et al. (2010) report that children with higher levels of adrenal androgens started breast development 0.8 years earlier, and pubarche 1.5 years earlier on average than those with a lower adrenal androgens. In a cohort study of Brazilian girls, those with premature pubarche caused by premature adrenarche entered thelarche and menarche before expected but within the normal range (de Ferran et al. 2011). The pattern of androgen production between adrenarche and menarche differed between Peruvian children living at sea level and at high altitude (Goñez et al. 1993). While both groups demonstrated two peaks of androgens—the first peak reflecting adrenarche and the second peak reflecting pubertal onset—the timing and tempo of these peaks differed by elevation. Both peaks in androgens levels were later among girls living at high altitude, and the interval between peaks was likewise longer, suggesting that a delayed adrenarche slows the tempo of pubertal development. These studies discussed here suggest that the intensity of adrenarche as marked by adrenal androgens is a modulator of pubertal timing.

Prenatal growth, often measured by size at gestational age, may also influence the tempo of puberty. Many studies have found earlier age at pubertal onset and menarche among girls born small for gestational age (Persson et al. 1999, Bhargava et al 1995, Ghirri et al. 2001, Ibanez et al 2000), while other studies have found no such associations (Veening et al. 2004, Albertsson-Wikland et al 1994, Jaquet et al. 1999). Some studies have suggested that the combination of being born small with subsequent rapid weight gain and growth during childhood is associated with the development of exaggerated adrenarche (Ong et al. 2004), precocious pubarche (Neville and walker 2005), and early menarche (Sloboda et al 2007).

The Relationship Between Adrenarche and Puberty

Together adrenarche and gonadarche account for the increased production of sex steroids as a result of the activation of both the HPA and HPO axes. There is debate whether these axes are exclusively separate processes or if adrenarche fine-tunes the timing of gonadarche. The predominant understanding is that, during adrenarche, adrenal androgen levels begin to rise while the gonadal axis remains quiescent. At gonadarche, typically two years later, the HPG axis activates and gonadal hormone levels of puberty rise (e.g. oestradiol) and their related external physical manifestations (e.g., breast development) appear (Felig 1987).

Sklar (1980) demonstrated that adrenarche and gonadarche are biologically separate occurrences controlled by separate mechanisms. He accomplished this by examining clinical situations where adrenarche occurred without gonadarche (gonadal dysgenesis syndrome and isolated gonadotropin deficiency) and gonadarche occurred without adrenarche (primary adrenal insufficiency and idiopathic precocious puberty) leading to the conclusion that they are separate processes. While reviewing these clinical cases proved helpful in establishing that adrenarche and gonadarche are regulated by separate mechanisms, a close examination

of the Sklar et al. study highlights that more research is needed. For example, among girls with idiopathic precocious puberty, the mean plasma concentration of DHEAS for patients between ages 1 –6 years did not differ significantly from that of normal pre-pubertal and pre-adrenarcheal children under 6 years of age. However, this finding is based on a small sample size (n= 11) and, descriptively, DHEAS levels are higher among patients (26.1 ± 7.8 vs. 11.1 ± 3.2 µg/dl) (Sklar et al. 1980). Sklar et al.'s (1980) study of children with pathological conditions is widely cited but it does not speak to natural variation in androgen and oestrogen levels among healthy juveniles.

In the same year that Sklar demonstrated separate processes for adrenarche and gonadarche, Vihko and Apter (1980) proposed that androgens may influence the onset of menarche. They suggest that a critical concentration of androgens affects follicular activity which leads to decreased steroid production by the ovaries. Results from a more recent study among patients with complete androgen insensitivity syndrome found a female pattern of puberty in individuals with an XY karyotype. This implies a major role of androgens in determining the timing of gonadotropin secretion at puberty (Papadimitriou 2006). Such findings propose that, in healthy populations, adrenarche and androgen production may be a separate but related process to puberty.

There is also confusion surrounding the relationship between adrenarche and pubarche. Many studies equate adrenarche with the presence of sexual hair growth (Biro et al. 2003; Zukauskaite et al. 2005; Idkowiak et al. 2011) because androgens mediate the growth of sexual hair. The number of hair follicles and the sensitivity of skin to androgens differ across individuals and partially account for ethnic variation in hair distribution (Archer and Chang 2004). Two individuals could have similar androgen levels but markedly different hair growth or sebaceous activity. This variation in skin sensitivity may explain some of the discrepancies in studies that use sexual hair growth as a marker of adrenarche. Some studies

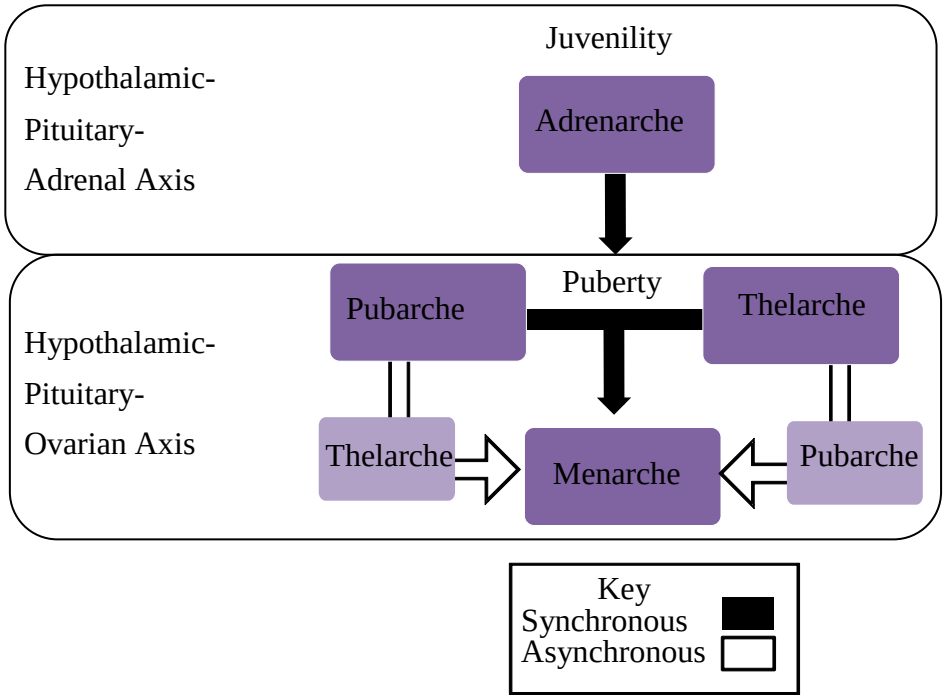
use the term adrenarche synonymously with pubarche. Much of the confusion between the terms comes from clinical studies of premature adrenarche, which is defined as the presence pubic hair before the age of 8 years in girls (Utriainen et al. 2007). Even though Wilkins (1948) first referred to this condition as premature pubarche, Talbot (1952) later re-termed the condition as premature adrenarche when androgens were speculated to be associated with the condition. Utriainen et al. (2009) best distinguish the terms from each other by defining pubarche as the clinical manifestation of androgen production. However, the terms remain in use interchangeably, though erroneously, in some studies.

The debate surrounding whether to include pubarche as a marker of puberty adds to the murkiness of understanding how adrenarche relates to puberty overall. Some researchers include pubarche as a marker of puberty in their studies (Marshall and Tanner 1969; Herman-Giddens et al. 1997; Huen et al. 1997; Wu et al. 2002; Juul et al. 2006), while others argue that pubarche should not be considered as a marker of puberty at all but rather as the manifestation of the HPA axis (Parent et al. 2003). I argue that pubarche is a part of puberty. From a practical point of view, pubic hair growth usually occurs during the pubertal transition and hair growth is seen as a physical sign of puberty to peers, parents and clinicians alike. After all, the term "puberty" is derived from the Latin pubescere, "to grow hair" (Bogin 2001). But, from a biological perspective, there is evidence that the HPO axis plays a role in the appearance of pubic hair. Girls with Turner Syndrome and primary gonadal failure had an earlier adrenarche and later pubarche than controls, suggesting that pubarche is the clinical manifestation of the ovarian conversion of DHEAS to active androgens (Martin et al. 2004).

While much of the debate, as summarized above, needs to be confirmed by experimental evidence, I propose a theoretical model of sexual development that includes adrenarche as well as gonadarche, and distinguishes adrenarche as separate from pubarche. Figure 3

illustrates the putative pathways of sexual development between adrenarche, thelarche, pubarche and menarche.

Figure 3: Theoretical Model of Sexual Development



The model above distinguishes adrenarche from pubarche and marks adrenarche as the first change to occur during juvenile development. A child could then progress from adrenarche to menarche either synchronously or asynchronously through either the pubarche or thelarche pathway. By positioning adrenarche in the sexual development model, it is now possible to compare how the timing of juvenility compares across groups of girls in a migrant study.

JUVENILITY IN A BIOCULTURAL MIGRANT STUDY

Migrant studies allow for researchers to distinguish between contributions of environmental and genetic factors to health and disease. International migrants re-establish themselves in a host country with an environment that often differs dramatically from their country of origin, not only physically but also socially and culturally. Because migration presents a unique

research opportunity that would not have been created intentionally, studies of migrant groups are often referred to as natural experiments.

Most migrant studies are based on the fact that the country of origin and host country differ markedly in relation to ecology, mortality and incidence of disease. Incidence rates among migrants tend to shift in the direction of the prevailing rates in the host country (Kolonel and Wilkens 2006). The change in outcome can be gradual over several generations or abrupt over the life course of one first generation individual. Therefore, it is important to distinguish between migrants and migrant groups. For the purpose of this study I use the following definitions as outlined by Pollard (2011):

- Migrants: those who have migrated within their lifetime from one country of permanent residence to another
- Migrant groups: encompass migrants and their descendants
- Migrant status: distinguishes between migrants and their descendants

To these terms I add the following:

- Migration scale: distinguishes among the population in the country of origin, migrants, migrant groups and native population in the host country from each other. These populations are ordered by individual/ancestral generations (IAG) lived in the UK.
- Migration groups: refers to the four groups including, Sylheti, first generation British-Bangladeshi, second generation British-Bangladeshi, and white British girls.

The genetic makeup of migrant groups (including first, second and third generations, and so forth where intermarriage has not occurred) is often similar to the population living in the country of origin. Ethnicity can be argued to be the same between the population of the country of origin and migrant groups but different between the migrant groups and the population of the host country (Kolonel and Wilkens 2006). First generation migrants experience considerable environmental disruption and experience a change in ecology. In contrast, descendants of migrants in the second and third generations do not experience country-level ecological discontinuity, but may undergo local-level changes in exposure to risk factors associated with migration, mediated by local-level ecology and lifestyle.

Many epidemiological studies employ migrant models in country pairs where the incidence of the outcome variable is higher among the host country compared to the country of origin. For example, Ziegler at al. (1993) compare breast cancer rates between Asians and Asian-Americans who had migrated to the US where breast cancer incidence is six-fold higher than in Asia. Each generation of individual or ancestral residence in the host country is a measure of exposure to risk factors associated with the higher breast cancer risk outcome. Following the predictions of a migrant model study design, second generation migrants groups would have higher breast cancer incidence than first generation migrants because first generation migrants would have experienced exposure levels of a lower risk country for a portion of their early lives. Similarly, third generation migrant groups would have higher incidence rates than the second generation because second generation migrant groups would have been more influenced by the cultural practices of the first generation migrants. If incidence rates for migrant groups reach the rates for host country population, then breast cancer can be attributed to environmental variables (Kolonel and Wilkens 2006). If residual difference persists after several generations in the host country, despite evidence of 'complete acculturation', one might conclude that a hereditary component, perhaps reflecting

differential susceptibility to the effects of environmental risk factors, contributes to breast cancer risk.

A particular advantage of migrant studies is their potential to provide insights into the period of life when relevant exposures have their greatest impact on adult health outcomes (Kolonel and Wilkens 2006). Identifying these critical periods is only possible by making comparison within first generation migrants who migrate at different times in their life course. The period of life when the influence of risk factors is greatest can be identified by comparing age at migration. Time elapsed since migration tests if the duration of time spent in the host country leads to greater exposure to risk factors in the new environment. Age at migration and time elapsed since migration are clearly related and caution needs to be taken when trying to tease apart their relationship (Parkin 1992).

There are limitations to a migrant model. The migrant model assumes that acculturation is a simple unidirectional process, rather than a dynamic process where social and biological factors interact in affecting behaviour and health. It also assumes that migrants are representative of the general population of the home country on which comparisons are based. A more accurate model would consider the social structures that affect behaviour and would recognise that acculturation may not be a one way road (Pollard 2011). Migrant status and the host environment could interact in an unexpected way and lead to an exaggerated response, which points to an additional contributing factor. A particular migrant group could have greater susceptibility to certain risk factors. If an exaggerated response persists, then migrant groups would have higher risks than the host population despite acculturation.

There are also limitations in using a migration scale to describe the various populations of girls recruited into the ABBY Project. Much of the literature on migration regards immigrants as being assimilated or acculturated into the host county (DeJaeghere and

McCleary 2010) and this is said to occur through a linear process. Some criticise this view of migration. When talking about the South Asian Diaspora, Hutnyk and colleagues (2005) suggests that the label of "migrant" is problematic as it suggests that people are not settled and therefore do not belong. Villenas (2007) suggests that rather than continuing to focus on assimilation, studies should "historicize" migration, "denaturalise" the "host" country, and focus on communities and identities in transnational spaces. While the connotations of "migrant" may hint at British- Bangladeshis history in the UK, these terms do not adequately describe the current situation of British-Bangladeshis living in the UK today. For the purpose of this study, the participants were classified by individual/ancestral time lived in the UK (migration scale), but the terminology used by the migration groups themselves will be explored later in Chapter 5.

RATIONALE BEHIND STUDY PREDICTIONS

The different ecologies of Sylhet, Bangladesh and East London, UK can be summarised as ecologically stressed versus ecologically abundant relative to each other. Food, sanitation and health care is more accessible in the UK than in Bangladesh, although socio-economic status mediates access to these services within both populations. The migrant population from Sylhet may be better off when compared to low socio-economic groups in Bangladesh, but worse off when compared to groups in the UK. A life history model would predict Bangladeshis maturing later than Britons.

The reasoning behind ABBY's predictions can be found in the literature pertaining to the relationship between early life factors and age at menarche. The secular decline in age at menarche, as observed in Europe, is attributed to the overall improvement in general health, nutrition, and living conditions that took place between the 1800s and mid 1900s (Euling et

al. 2008). A comprehensive literature review of 30 studies investigating associations between early life factors and age at menarche found consistent evidence that earlier menarche was associated with larger body size, better social circumstances, and exposure to unfavourable psychological circumstances (Mishra et al. 2009). Specifically, birth weight, childhood adiposity and socioeconomic status are negatively correlated with age at menarche (Billewicz et al. 1983, Cooper et al. 1996, Persson et al. 1999, Adair 2001, dos Santos Silva et al. 2002, Anderson et al. 2003, Romundstad et al. 2003, Chavarro et al. 2004, dos Santos Silva et al. 2004, Ersoy et al. 2004); whereas gestation length, duration of breast-feeding, and experience of acute and chronic illness are positively correlated with age at menarche (Bhargava et al. 1995, Kim & Smith 1998, Tahirovic 1998, Khan et al. 1996, Rosenstock et al. 2000, Novotny et al. 2003). Therefore, it can be predicted that Sylheti girls will mature later than white British girls because they are relatively smaller at birth (Davies et al. 1989), breastfed longer (Núñez-de la Mora 2005), less obese (de Onis et al 2012), and experience more acute and chronic illnesses (Bern et al. 1992).

It can also be predicted that migrant girls will mature earlier than Sylheti girls due to changes associated with migration that occur during development, such as increased energy available for growth and development. Studies with Scandinavian families adopting Asian children have found that adopted children mature earlier than girls living in the host country or the country of origin (Proos et al. 1991, Teilmann et al. 2007).

Previous studies conducted with Bangladeshi migrants to London collectively suggest that migration to the UK as a child, specifically prior to puberty is associated with elevated adult reproductive hormone profiles that are either comparable with or higher than white British adults (Núñez-de la Mora et al. 2006, Magid 2011, Begum 2011). For example, among premenopausal women, luteal phase progesterone levels were significantly lower among

Sylheti and adult migrant women when compared to levels among child migrants, second generation migrants, and white British women (Núñez-de la Mora et al. 2007). Among post-menopausal women, Sylheti and adult migrants have lower age-specific ovarian reserve (as marked by lower levels of inhibin B and anti-Müllerian hormone and higher levels of follicle-stimulating hormone) than white British and child migrants (Begum 2011). In men, recalled age at maturity was older and adult testosterone levels were lower in Sylheti and adult migrants compared with child migrants, second generation and high status white British men (Magid 2011). In all of these studies, reproductive hormones of adult migrants did not differ from hormones measured in the Sylheti groups, whereas most reproductive hormones of child migrants were higher than those measured in Sylheti groups. In some instances, hormones among child migrants were even higher than in the white British comparison groups. These collective findings suggest that there is a critical window during childhood which affects adult reproductive function.

It is possible that there are two models at play in relation to how migration between countries with different ecologies affects adult reproductive function. Across migration groups there is an effect of intergenerational cumulative exposure to the ecology of the UK on hormone levels while, within the first generation migrants, there is an added effect of lifetime cumulative exposure to the UK within a critical window. If a girl migrates at a young age before sexual maturation, then her reproductive steroid hormone levels are predicted to be higher than a girl who did not migrate or who migrated after puberty. Her hormonal levels could also be higher than her second generation and white British counterparts showing an exaggerated response to the exposure to ecological conditions in the UK before the age at maturity.

ABBY is designed to test whether the discontinuity in ecological conditions that would be experienced by first generation migrants is associated with the timing of juvenility. The first generation migrants in my study are comparable to child migrants in prior Bangladeshi migrant studies; thus, the predictions of ABBY are derived from the patterns found in the Núñez-de la Mora et al. (2007) study and recent unpublished PhD theses (Begum 2011, Magid 2011). In order to test the critical window hypothesis adequately, age at migration and time since migration should be considered for first generation migrants. However, the small number of participants in the first generation group limits my study's ability to test for the presence of a critical window during childhood (see Chapter 2).

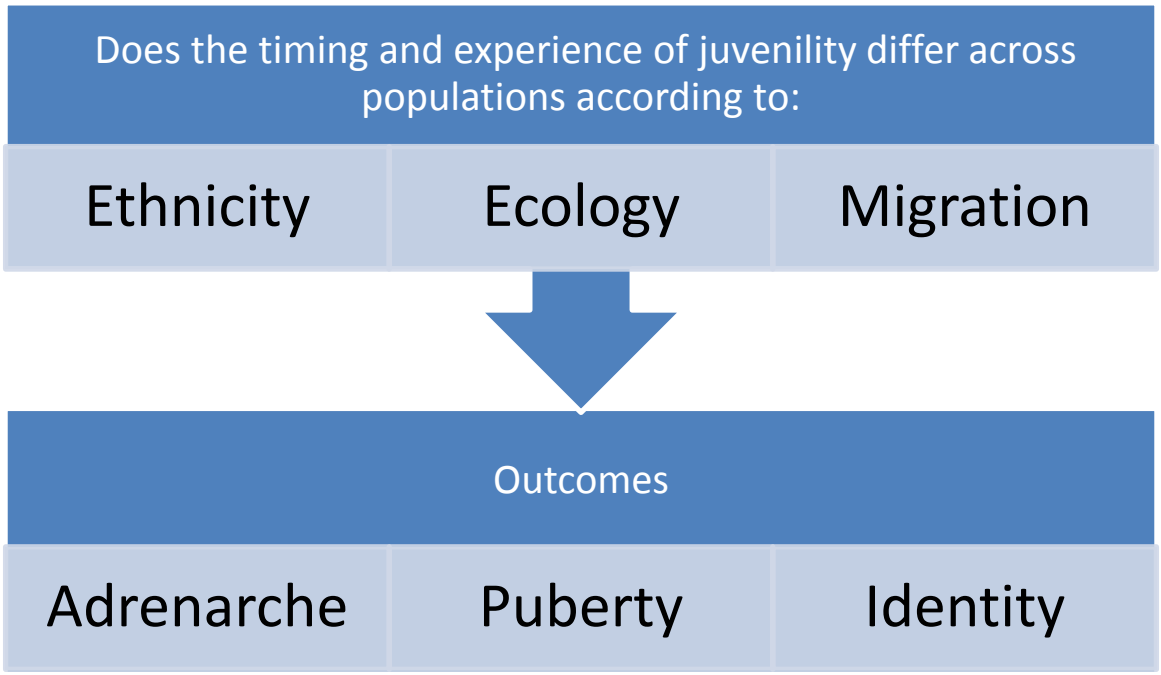
STUDY DESIGN AND HYPOTHESES

The ABBY Project explored age differences, hormonal variation and lifestyle factors associated with the juvenile period. It is a migrant study that collected individual-level data from girls and information on the wider cultural context to address whether juvenility varies by ethnicity, ecology and migration. To answer this question, ABBY's research objectives were twofold:

- To assess whether there is variation (biological and cultural) in juvenility across populations
- To identify the determinants of juvenile onset by comparing energetic (diet and physical activity), psychosocial, immunological and epigenetic factors.

However, the scope of the ABBY Project is very wide, and so I focus on the first objective (Figure 4) in this thesis by testing if the timing and experience of juvenility differs by ethnicity, ecology, and migration.

Figure 4: Objectives and Variables of the ABBY Project



In this thesis I explore this objective by following these aims:

- To determine whether juvenility differs by ethnicity, I compare the timing of adrenarche and puberty between all Bangladeshi and white British girls.
- To determine whether juvenility differs by country-level ecology, I compare the timing of adrenarche and puberty between all girls living in the UK to Sylheti girls. To determine if juvenility differs by discontinuity in country-level ecology I compare first generation migrant girls to Sylhetis.
- To determine if juvenility differs by migration, I compare the timing of adrenarche and puberty among Sylheti, first generation British-Bangladeshi, second generation British-Bangladeshi, and white British girls according to the migration scale. I also compare the experience of growing up among all four migration groups.

The overarching hypothesis is that juvenility will differ among populations according to a mixture of cumulative exposure to and discontinuity in country--level changes in ecology.

Hypotheses based upon the life-history model are tested according to methodology detailed in Chapter 2 and presented in three results Chapters. Chapters 3 and 4 explore the endpoints of the juvenile period as defined by biological markers. Chapter 3 will focus on adrenarche: the transition from childhood into juvenility. Chapter 4 investigates puberty: the transition from juvenility to adolescence. Chapter 5 considers growing up in the context of migration and explores juvenility using cultural markers.

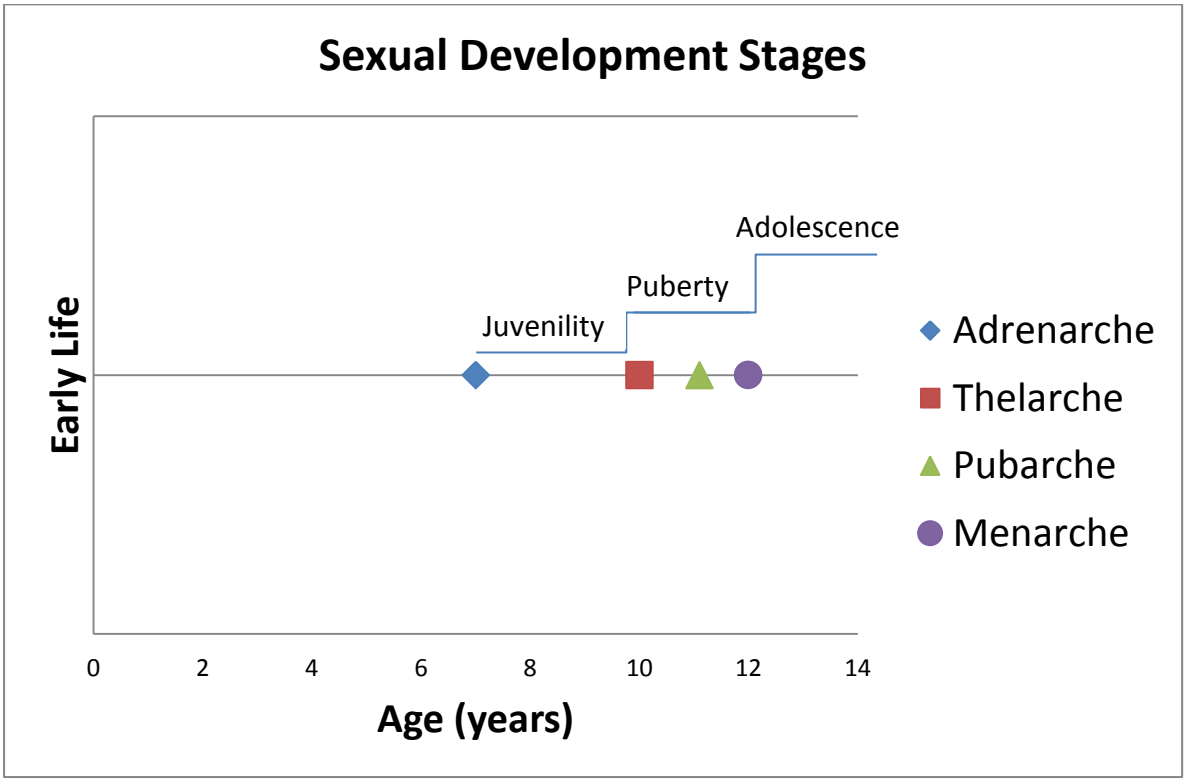
In Chapter 3 I test whether age and size at adrenarche differ by ethnicity, ecology and migration status. The specific predictions are that white British and second generation British-Bangladeshi girls who spent all of their lives in London will reach adrenarche at an earlier age, be taller and fatter at this time, report more somatic signs of adrenarche at an earlier age, and show higher DHEAS levels than Bangladeshi girls who live in Sylhet. Girls who spent part of their lives in Bangladesh before moving to the UK will reach adrenarche earliest, be tallest, fattest, report somatic signs of adrenarche earliest and show the highest levels of DHEAS.

In Chapter 4 I test whether age and size at pubertal onset and maturation differ by ethnicity, ecology and migration. The predictions are that white British and second generation British-Bangladeshi girls who spend all of their lives in London will reach thelarche, pubarche and menarche earlier, be taller and fatter at these times and will show higher oestrogen levels than Bangladeshi girls who live in Sylhet. Girls who spent part of their lives in Bangladesh before moving to the UK will reach thelarche, pubarche and menarche earliest; be tallest and fattest, at these times and will show the highest levels of oestrogen levels. The second part of Chapter 4 compares the interval between adrenarche and pubertal stages across migration groups. The prediction is that the order and tempo of juvenile development will be similar across all migration groups due to similar phylogenetic constraints present in all girls.

Chapter 5 explores the experience of growing up as a British-Bangladeshi girl in London. I explore whether acculturation is a unidirectional process for Bangladeshi migrant groups to the UK and whether the psychosocial experience of growing up in the East End of London runs parallel with biological development.

Chapter 6 discusses the findings at two levels of explanation—proximate and ultimate—and then contextualises the findings of ABBY within public health.

Figure 5: Theoretical Age and Relationship Among the Stages of Development Measured in ABBY



The above schematic illustrates the early life segment of the human life course. Each shape represents a different early life stages and the stepwise line illustrates the juvenile and pubertal intervals between them. These stages are plotted by ages that the current literature suggests as 'normal' for each stage of development. At the end of each Chapter, I will provide an interlude with additional schematics that build upon this model using data from the ABBY Project.

CHAPTER 2: STUDY POPULATION AND METHODS

SUMMARY

This Chapter describes the methodology used in the ABBY (Adolescence among Bangladeshi and British Youth) Project. This Chapter begins with the theoretical approaches to biocultural and mixed-methods research and illustrates how they were implemented. The majority of the methods for ABBY took place during two and a half years of fieldwork. Historical, demographic, economic and pre-existing ethnographic details about the field sites are described. ABBY adapted prior methodology to be implemented with the populations under study and these methods were tested and validated during the pilot phase. A detailed recruitment procedure is described that follows from the population level down to the individual. Quantitative and qualitative data collection methods are reported and the laboratory analyses are described. Conducting biocultural research with children was an iterative process and this Chapter includes my engagement with the research process itself. The selected methodology described in detail in this Chapter builds upon validated methods used in prior studies in anthropology, reproductive ecology and epidemiology. Therefore, I adapted prior methods, introduced novel methods specific to assessing adrenarche, and triangulated biological data with ethnography and demographics to answer ABBY's research objectives. To my knowledge, ABBY is one of the first studies to employ such methods with children growing up in England and Bangladesh.

INTRODUCTION: BIOCULTURAL, MIXED METHODS RESEARCH

The ABBY Project explores the relationship between migration and growing up from a biocultural perspective. Biocultural research includes social, cultural or behavioural variables in addition to biological measures in its study design (McElroy 1990). There are both integrative and segmented approaches to biocultural methods (ibid). An integrative approach equally weighs sociocultural, biological and environmental data, whereas a segmented approach primarily collects biological data while cultural and environmental factors are considered, but only peripherally. Some of ABBY's aims required data that could be measured precisely and statistically, requiring a certain sample size and demographic. Other aims explored the experience of growing up by understanding the subtleties and contradictions that arise in daily lives of the participants.

In relation to conducting research on childhood, James, Jenks and Prout (1998) pose the question whether one should emphasize childhood as structure or children as agents. My goal in designing ABBY was to integrate measures of childhood development with observations of children as agents in their daily lives (and, by default, in the research process). ABBY's mixed methods approach allowed for this integration.

Mixed methods research combines research methods that cross both quantitative and qualitative research strategies. My approach to mixed methods in this study can be described by "diversity of views" and "unexpected results" (Bryman 2008), both of which allowed me to gain access to the perspectives of growing up among the participants and to explore specific questions regarding the timing of juvenility. The quantitative research methods were deployed to address specific hypotheses, while more open-ended qualitative methods were designed to allow novel findings to emerge.

As McElroy (1990) acknowledges, it is easy to include both biological and cultural factors in study design, but it is more difficult to simultaneously and systematically collect data. During fieldwork, I employed quantitative and qualitative methods often in separate contexts, although the findings of one fed into the execution of the other in an iterative process.

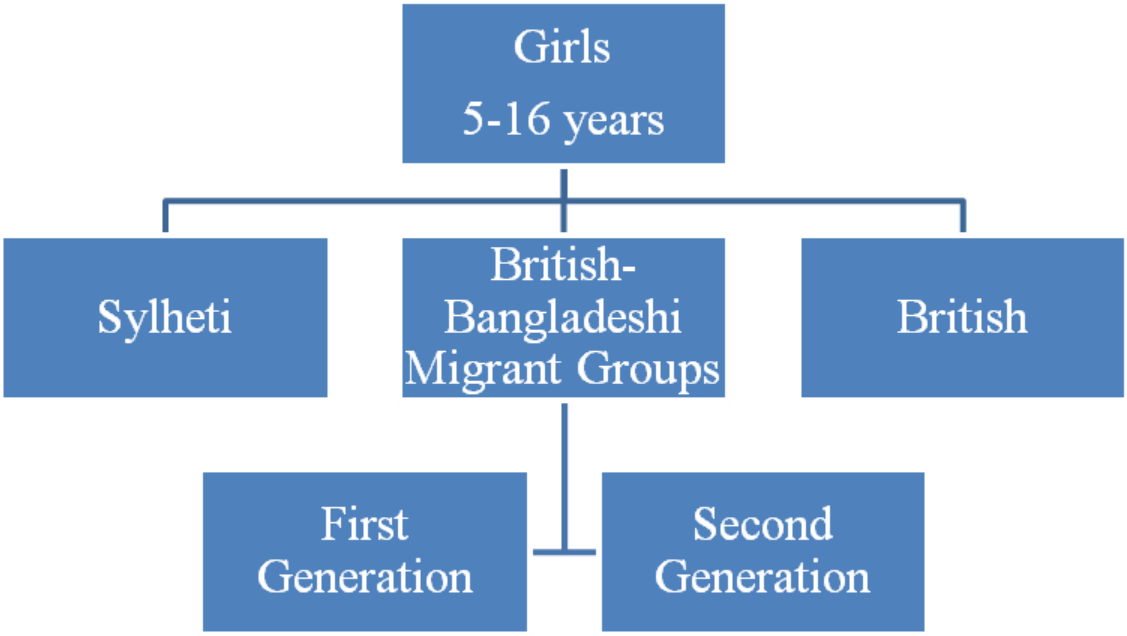
When presenting the results, I focus on data derived from the quantitative methods in Chapters 3 and 4, while in Chapter 5 I focus on qualitative data regarding migration.

Chapter 5 and the final discussion Chapter also employ triangulation. Triangulation implies that results derived from one research strategy are cross-checked against the results of another research strategy (Bryman 2008). In Chapter 5 I triangulated acculturation data when comparing questionnaire data regarding ethnicity and identity with themes that emerged during focus groups. In the final discussion Chapter I combine both quantitative and qualitative strategies to explain the main findings of ABBY. It is my intention that any deviations from the integrative approach that took place during fieldwork are less segmented through the analysis and synthesis of the biological and cultural data in the final discussion Chapter.

STUDY DESIGN

Mixed methods were used in a cross-sectional study to collect both biological and cultural data surrounding the experience of juvenility among Sylheti, British-Bangladeshi and British girls aged 5 – 16 years across two field sites: London, UK, and Sylhet Town, Bangladesh.

Figure 6: Cross-Sectional Study Design of Sylheti, British-Bangladeshi Migrant Groups and British Girls aged 5-16 years



PARTICIPANT CLASSIFICATION

All participants were classified into one of four study populations (Figure 6):

- Sylheti (S): girls who were born and living in Sylhet, Bangladesh to parents of Bangladeshi ancestry
- First generation British-Bangladeshi (BB1): girls who were born in Sylhet, Bangladesh to parents of Bangladeshi ancestry and were living in the UK at the time of participation
- Second generation British-Bangladeshi (BB2): girls who were born and living in the UK whose parents were of Bangladeshi ancestry with at least one parent who was born in Bangladesh.
- White British (WB): girls who were born in the UK and whose parents were also born in the UK of white European ancestry.

These classifications were based on a child's place of birth, parents' place of birth and self-reported ethnicity. Some of the second generation British-Bangladeshi girls had one parent born in the UK and one parent born in Bangladesh. Some British girls (including all ethnicities) were born to parents who were born outside of UK and/or are not of white European ancestry. This population was excluded from all analyses because their ancestral histories were varied.

Differences among the four study populations were tested using pair-wise and trend analyses. The trend analyses tested for variation in the outcome variables according to migration scale, so a dummy variable based on individual/ancestral generations lived in the UK (IAG) was created. Sylheti girls were the reference population and the subsequent populations were ordered by increasing individual/ancestral generations lived in the UK. Migration scale was coded 0 to 3 according to the number of generations lived in the UK as follows:

0 = Sylhetis (Bangladeshi subject and her parents have only lived in Bangladesh; no individual or ancestral generations in the UK)

1 = First generation British-Bangladeshi (Bangladeshi subject was born in Bangladesh and has lived in the UK; her parents were not born in the UK; one individual generation in the UK, no ancestral generations)

2 = Second generation British-Bangladeshi (Bangladeshi subject was born and has lived in the UK and at least one parent was born in the UK; one individual generation, one ancestral generation in the UK)

3 = White British (White subject and her parents all born in the UK; one individual generation, more than two ancestral generations in the UK).

I acknowledge that there is debate revolving around the inclusion of a white sample in studies that research populations of ethnic minorities. Some argue that the inclusion of a white sample inherently sets up a deficit perspective of non-whites to whites (McLoyd 1991). But as Syed (2012) explains, the decision to include a white comparison group depends on the study design:

> On the one hand if researchers are interested in documenting that a given aspect of development is different in some way between one or more ethnic minority groups and Whites, then a White sample would be essential to include. However, if researchers are interested solely in the descriptive or phenomenological experiences of a particular group, or want to examine individual differences within an ethnic group—and make no claims to uniqueness or differences between groups—then a White sample is not necessary (p. 10).

The objective of this study is to establish if there is variation in the experience and timing of juvenility across populations. Up until now, adrenarche has only been studied in white populations; therefore, ABBY is unique in that it is measuring the age at adrenarche among Bangladeshi, British-Bangladeshi girls and British girls. In this study the white British girls

are ethnically different from British-Bangladeshi girls, but experience similar ecologies in East London. Measuring adrenarche in white British girls who attend the same schools as their British-Bangladeshi counterparts allows for internal comparison between subjects in one study and for cross-comparisons with pre-existing studies conducted with children of European ancestry. Finally, patterns among all four of the migration groups included in my study can be compared to the same populations studied previously by Bentley and her colleagues. My study design allows for the documentation of variation in juvenile development across populations, so that population characteristics specific to different ecological environments can be viewed as adaptive to context rather than in deficit to other populations.

STUDY SETTING: LONDON, UK AND SYLHET TOWN, BANGLADESH

Fieldwork occurred during the months of September 2009 until December 2010 in England's capital city, London; and then from January 2011 until April 2011 in Bangladesh's north-eastern division capital, Sylhet Town. Comparing Sylheti and British-Bangladeshi girls in a migrant model is an ideal situation in which to ask whether the developmental environment is associated with the timing of juvenility because 95% of British-Bangladeshi migrants originate from Sylhet (Gardner 1992). They are almost uniformly Muslim and intermarriage is rare (Gardner and Shukur 1994). Also, the developmental environment in Bangladesh differs greatly from that in the UK, specifically with regard to access to health services and exposure to infectious diseases which are both likely to affect childhood development. Migrants come from the landowning class with relatively high socioeconomic status in Sylhet (Gardner and Shukur 1994), meaning they are more comparable to children in the UK than to poor Bangladeshi girls.

Migration from Sylhet to London has been well established for over 100 years. There is evidence for this as early as the 1850s when seafaring men known as *lascars* arrived on East Indian Tea Company ships (Communitites and Neighbourhoods 2009). Later in the 19th century, men seeking higher education also migrated to the UK. By the 1950s, India had been partitioned, Bangladesh was East Pakistan and British officials sought workers to staff London's post-war boom. By the 1960s migration from Sylhet increased and mainly consisted of men entering on the voucher system established by the 1962 passage of the first UK Commonwealth Immigrants Act.

When the "myth of return" (Anwar 1979), the notion that being outside of one's country of origin is temporary, was replaced with the realities of settling in the UK, men started to bring their wives and children over to build a life on British ground. More and more Bangladeshi

family members immigrated to London in the 1970s when Bangladesh was fighting for independence from Pakistan. Political instability pushed emigration from Bangladesh, while the promise of family reunification pulled immigration to the UK. Women and children immigrated to be reunited with their husbands or fathers. Nowadays, the migration pattern has shifted again with stricter UK immigration policy and marriage being the main mechanism for migration. Today, Sylheti newlyweds- women or men- migrate to the UK on a spousal visa and start a family with their British national spouse (Gardner 1993). Most new immigrants from Bangladesh are between the ages of 15 and 30 years (Mayhew and Harper 2010).

Figure 7: Map of England

Source: http://www.lonelyplanet.com/maps/europe/england/

To understand better the environment where British-Bangladeshis live today, it is helpful to explore the current demographics in east London. UK immigration data indicate that 154,201 Bangladeshi-born people lived in the UK in 2001, which is a 50% increase since 1991 (IPPR 2005). In 2009, dependents accompanied 13% of Bangladeshi immigrants (Achato et al.

2011). In 2001, 65% of Bangladeshi migrants earned less than half the UK median wage, indicating that Bangladeshis are economically the poorest migrant group living in Britain (IPPR 2005). The majority of British-Bangladeshis live in the London Borough of Tower Hamlets. The following borough statistics were based on a school census carried out in 2001 and were later adjusted in 2009 (Mayhew and Harper 2010):

- Tower Hamlets is home to 55% of Britain's Bangladeshi-born community and makes up 1% of London's overall population as of the 2001 census.
- Within the borough, 32% of inhabitants are Bangladeshi, comprising the largest fraction of all ethnic groups there (ibid).
- The Bangladeshi population totals 75,401 people, closely followed by the white British/Irish population of 64,284 people, which comprises 27% of the total population.
- Of all children in Tower Hamlets aged 0 – 19 years, Bangladeshi children account for 55.2%.

Tower Hamlets is an economically deprived area of London with 43% of all households receiving means tested benefits and 62% of children in Tower Hamlets living in such households. Furthermore, 73% of all Bangladeshi residents receive means tested benefits. Deprivation is unequally distributed according to ethnicity and age. In Tower Hamlets, a person who is Bangladeshi, aged 16 – 24 years, and living in social housing has 73 times higher odds of qualifying for a council tax benefit than if a person is associated with none of these factors (Mayhew and Harper 2010).

Figure 8: Map of Bangladesh

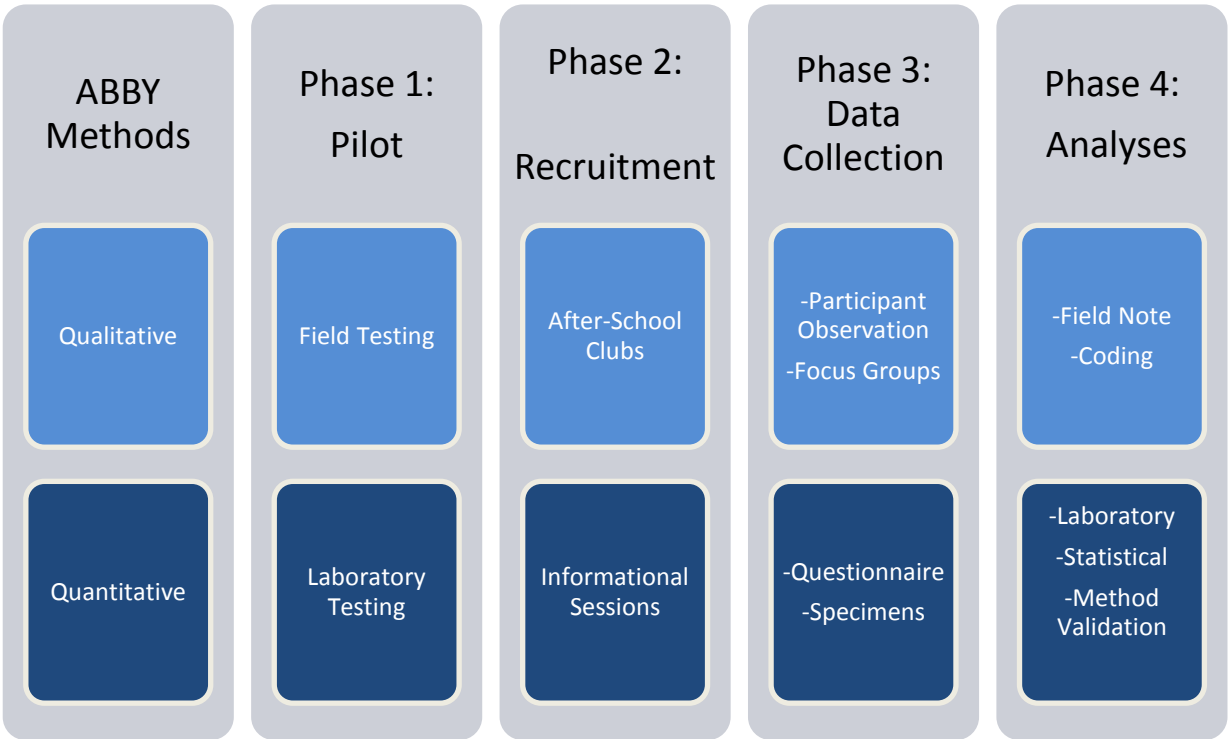
Source: http://www.lonelyplanet.com/maps/asia/bangladesh/

It is important to distinguish between the different populations within Sylhet (Figure 8) and to acknowledge that the pattern of migration from and within Sylhet has shifted over time. There are fewer families moving directly from the villages to the UK; more rural families are choosing to move to urban areas such as Sylhet Town as Sylhet's economy grows. The World Bank reports that in urban centres, including Sylhet, 10% of migrants are from the UK and 18 % are domestic migrants (Sharma and Zaman 2009). The rural-to-urban migration happening within Sylhet itself is changing the demographics of this most densely populated part of the most densely populated country in the world.

ABBY Project Phases

The ABBY Project consisted of four distinct research phases—Pilot, Recruitment, Data collection and Analyses. Within each phase the methods can be separated into quantitative qualitative research strategies (Figure 9). In the pilot phase, field testing was performed to assess feasibility of the proposed methods and laboratory testing was conducted to validate the salivary and urinary assays of biomarkers. The second phase consisted of recruiting schools and enrolling girls attending these schools into the study. The third phase involved administering questionnaires, collecting biological samples and participating in participant observation, including after-school clubs and focus groups. The final phase involved the analyses of data including the following three stages: laboratory analyses of the biological samples, method validation, statistical analyses and coding of field notes.

Figure 9: The Mixed Methods Employed during the ABBY Project's Four Phases



PHASE 1: PILOT

FIELD TESTING

The field testing pilot began in June 2009 when I set out to answer the following questions:

- Which methods best answer the study objectives?
- For which methods will girls and their families provide consent?
- Which methods are possible during a school day and over a school year?

During the pilot phase of fieldwork, I spoke with 50 girls and 15 mothers living in the London area about the methods that ABBY was to employ later in the year. Through participant observation of a school day at two schools and three focus groups in schools and community centres, I discussed the proposed methodology of ABBY, including body measurements, specimen collection and girls' and their mothers' understandings about puberty.

Body measurements: Some girls expressed hesitation about being weighed; they stated that if a girl was overweight she may not want be weighed. Such statements indicated that the main study might be biased towards those girls who are within the normal weight range because girls who see themselves as overweight may have chosen to opt out of the study altogether. A teacher's aide also expressed concern about weighing girls because, in her school, many of the Year 6 girls (aged 10 – 11 years) were sensitive about their weight. In the main study, all girls were asked to allow their weight and height to be measured but, like all aspects of the study, they were able to opt in or out of this particular section. Of the girls who completed interviews, 86% had complete anthropometric measurements taken.

Specimen collection: Some girls expressed disgust when asked about giving urine samples. I soon discovered that many girls had never provided urine samples and this made such a

request seem strange and unfamiliar. Those girls who had previously provided urine samples at a general practitioner's office were more familiar with the concept. As a result, future recruitment strategies included an in-depth description of why and how the urine samples were to be collected. Explaining how the samples were to be collected and transported in discreet packaging improved the participation rate of specimen collection.

Puberty: Initially, it was unclear whether the Pubertal Development Scale (PDS) would be an appropriate tool for measuring physical changes that occur during puberty. Petersen et al. (1988), first created the PDS and reported that this tool was effective in determining pubertal stages when compared to the Tanner stages; however, there is no published validation of this scale with the Bangladeshi populations being studied here. While a formal validation (which would require a physician to physically inspect pubertal staging) was not carried out, it was deemed (through participant observation during the pilot phase) that answering questions about physical changes would be culturally appropriate with both British and British-Bangladeshi girls. (Hormonal validation of the PDS was later conducted in Phase 4). I observed a biology class in a secondary school where the teacher asked students to complete an assignment regarding pubertal development. The aim of the exercise was to match the correct pubertal term with the corresponding body part as illustrated on a hand-out. I sat with four British-Bangladeshi girls who became uncomfortable during the exercise and chose not to fill out the diagram. Noticing their lack of participation, I asked them verbal questions about the different stages and they preferred to answer my questions rather than complete the hand-out. As a result of this observation, ABBY Project interviewers asked the PDS questions, with the option to self-complete the PDS.

Mothers: During the pilot phase, I spoke with parents to gauge their perception of ABBY. I spoke to mothers of girls who participated in the focus groups. One mother thought the word "adrenarche" seemed threatening because it sounded like "anarchy" and suggested that I use

an alternative word. I then changed the A in the ABBY acronym from "adrenarche" to "adolescence" in the informational materials. I also spoke to mothers through a community centre for women and I observed a weekly public speaking class over the course of six weeks. I coordinated with the instructor of this course so that one of the debate topics was "Should a mother let her child participate in the ABBY Project?" The mothers said that the word "research" has bad connotations and so it became important to explain why ABBY is important and to offer an incentive, such as free BMI screenings for parents. Mothers also told me that asking their young daughters about puberty seemed premature. One mother told the group that she had reached menarche without ever being told what it was. Although she remembered feeling scared when she had her first menses, she would do the same for her daughter and not teach her about menstruation. Not all mothers agreed; one mother expressed how she would like to know more about puberty so that she could answer her daughter's questions. Another mother who asked her daughter about ABBY at home later wrote to me:

> *When I asked if [my daughter] would mind being asked personal questions she giggled. I put it in a jokey way, "What about when your boobies start growing?" and she seemed to think that would be ok - though she didn't know as it hadn't happened to her yet and I'd have to ask older girls! She thought younger girls might not understand what it was all about, but that from her age (7) they would be fine with it. It actually surprised me how grown up she was in discussing it, just goes to prove you can't underestimate them!*

After gaining insight into mothers' perception of ABBY, it was evident that most mothers saw the potential importance of the research. Discussing ABBY with mothers also became good practice for how to explain the project to future participants and their parents. Discussing the study with girls and mothers and conducting small parts of the proposed methods with them indicated that the large scale study would be feasible.

LABORATORY TESTING OF SALIVARY DHEAS AND URINARY OESTROGENS

During the pilot phase, saliva and urine specimens were collected from Bangladeshi and British girls for preliminary methods validation. While studies have been published that measure hormones during pubertal changes (Korth-Schutz et al.1976; Apter and Vihko 1985; Goñez et al. 1993; Blogowska et al. 2005; Remer et al. 2005; Taha et al. 2005; Maliqueo et al. 2009; Shi et al. 2010), none have specifically assayed androgens and oestrogens hormones in urine and saliva in Bangladeshi populations under study. This section of the pilot was designed to test if androgens and oestrogens were detectable and quantifiable from specimens provided by Bangladeshi and British girls.

During the pilot phase, saliva samples were collected from 30 British-Bangladeshi and white British girls aged 5 – 16 years. Unstimulated saliva was frozen until analysed for DHEAS via commercially available enzyme-linked immunosorbent (ELISA) assay kits (Salimetrics©). The 30 specimens were assayed in duplicate across two plates. DHEAS was detectable in all samples. The pilot results suggested that the ELISA would be a suitable assay to determine variation in salivary DHEAS levels across the study populations.

While Bentley and colleagues have previously measured salivary oestradiol among the Sylheti and British-Bangladeshi populations, it was not clear if salivary assays would be sensitive enough to detect childhood levels of oestrogens which would be presumably lower than premenopausal women. Shi et al. (2010) have previously measured levels of five urinary oestrogens and oestrogen metabolites in children while other metabolites were below their assay's limit of detection. My mentors at the National Cancer Institute have been instrumental in the successful development of a highly sensitive technology to measure 15 oestrogen and oestrogen metabolites in urine (Xu et al. 2005), so they were interested in applying their assay to child samples. It is important to note that oestrogens in saliva and urine do not reflect the same biologically relevant analyte. While saliva contains unbound

oestradiol which is not bound to sex hormone-binding globulin (SHBG), both conjugated and unconjugated oestradiol and its metabolites are measured in urine. Therefore the sum of all 15 oestrogens and oestrogen metabolites would better reflect circulating levels of oestrogen (Shi et al. 2010) rather than the unbound form found in saliva.

The urine pilot explored the ability of the liquid chromatography-mass spectrometry (LC-MS/MS) to detect endogenous oestrogens and oestrogen metabolites (jointly referred to as oestrogens) during adrenarche. The laboratory concurrently measured 15 oestrogens in specimens from Bangladeshi girls aged 5-9 years (n=10), British-Bangladeshi girls aged 5-9 years (n=10), and white British girls aged 5-9 years (n=10). The laboratory also measured oestrogens in Bangladeshi girls who were aged 12 – 14 years (n=10) in case levels were below detection for the younger girls. I designed the pilot to assess the limit of detection and the limit of quantitation (LOQ), and to estimate the sample size and power necessary for our future main study.

Limit of detection: The samples were separated into two batches; samples from British-born girls (including British-Bangladeshi and white British) were batched in one plate and samples from Bangladeshi-born girls in the other. Each sample was separated into two blinded aliquots and randomized onto one of the two plates. Within-batch coefficients of variation were calculated from these two samples. All 15 oestrogens were detectable for all samples in the study. All coefficients of variation were below or equal to 3% for each oestrogen.

Limit of quantitation: Concentrations of samples were compared to the published limit of quantitation to determine if the assay could reliably quantify low levels of oestrogen. The assay detected all 15 oestrogens with excellent within-batch CVs suggesting that all samples were above the LOQ.

The pilot indicated that ELISA and LC-MS/MS methods were able to detect and quantify all androgens and oestrogens in the pilot samples. Therefore, the large scale study could generate adequate information to make conclusions about potential cross-cultural differences in juvenile hormones.

PHASE 2: RECRUITMENT

SAMPLING TECHNIQUE

Girls, aged 5 – 16 years, were recruited from the general population via primary and secondary schools. I recruited girls from the general population rather than recruiting from a clinical population that could have biased the sample to pathological states. If a participant was taking hormone-based medication, this was reported in the structured interview and her biological samples were not collected or analysed. I used convenience sampling, which involves selection on a first-come basis within a predefined population, to recruit participants (Bryman 2008). Schools and girls were not sampled randomly and any conclusions from the study cannot be applied to the whole population from which the sample was drawn. No students were specifically excluded based on ethnicity. As a result, some girls coming from backgrounds other than British or Bangladeshi participated in the research but were excluded from statistical analyses. Figure 10 gives an overview of the recruitment process.

SCHOOL RECRUITMENT

In east London, there are three specific areas within Tower Hamlets where most Bangladeshi households are located, and schools were targeted in these areas. Overall, ten schools agreed to participate in the ABBY Project, consisting of two secondary and eight primary schools. In many of these schools, at least 50% of the students came from Bangladeshi households indicating that ABBY recruited from an area densely populated with British-Bangladeshis (Table 3). Initially, schools were contacted via drop-in visits and the distribution of written

project materials (Appendix 1). From this initial recruitment effort, two schools (UK Primary School 1 and UK Secondary School 1) agreed to participate. Additional primary schools were recruited through snowballing: key contacts at one school referred me to key contacts at other schools. UK Secondary 1 belonged to a partnership of schools that consisted of several primary schools designated as feeder schools; four of these schools (UK Primary School 2, 3, 4 and 5) agreed to take part in the study. A personal contact helped to recruit the other secondary school (UK Secondary School 2). All head teachers of the schools granted permission for their school to collaborate with ABBY, but many times the head teachers appointed another representative from the school as the key contact person for all subsequent interactions (Figure 10).

Figure 10: ABBY Recruitment Protocol

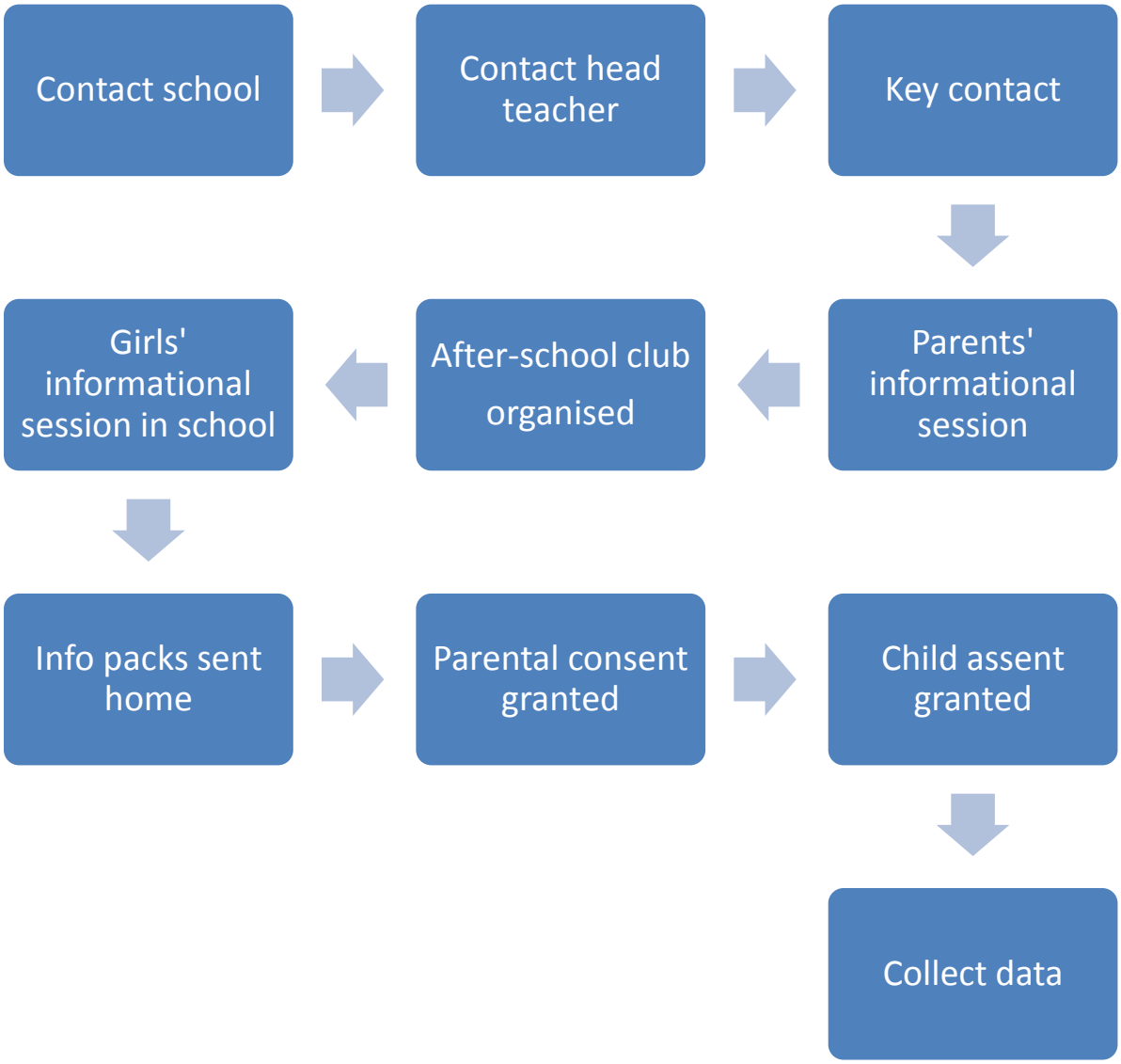


Table 3: Sex, Socio-economic Status and Ethnicity Statistics of the Participating Schools in the ABBY Project Schools

Phase of Education	School Name	ABBY-Enrolled Girls	Headcount of Total Girls	Headcount of Pupils	Percentage Eligible for Free School Meals	Percentage of White British	Percentage of Bangladeshi	Percentage of English as First Language
Schools in London*		296						
Primary	PS1	6	179	304	45.0	NA	NA	40
Primary	PS2	40	255	533	50.1	8.8	71	81.2
Primary	PS3	10	204	451	56.2	8.7	70.1	83.8
Primary	PS4	52	243	492	56.7	18.3	58.6	66.1
Primary	PS5	50	121	245	58.4	8.3	75	91.1
Primary	PS6	22	131	261	43.7	24.6	46.9	62.9
Primary	PS7	30	94	215	40.9	35.5	30.3	36.8
Secondary	SS1	45	423	880	66.3	27.4	51	63.3
Secondary	SS2	41	329	48	71.1	1.1	86.2	97.3
Schools in Sylhet		192						
Both	CS1	31	NOT AVAILABLE				100	NOT AVAILABLE
Both	CS2	30					100	
Primary	PS8	16					100	
Both	CS3	17					100	
Primary	PS9	23					100	
Primary	PS10	45					100	
Primary	PS11	30					100	

PS = Primary School
SS= Secondary School
CS = Combined primary and secondary school
*UK School Statistics according to 2009 Census (Statistics 2009)

In Sylhet, it was important to identify girls who belong to families that have a history of migration to the UK or who were most similar to other families that do. Registered private and semi-private/semi-government schools were targeted. These schools consist of students from middle to lower-middle class families that can afford to send their children for private education. Research assistants and I approached individual schools through drop-in visits and personal networks. Out of the eight schools approached, seven schools opted to participate in ABBY (Figure 11).

Participant Recruitment

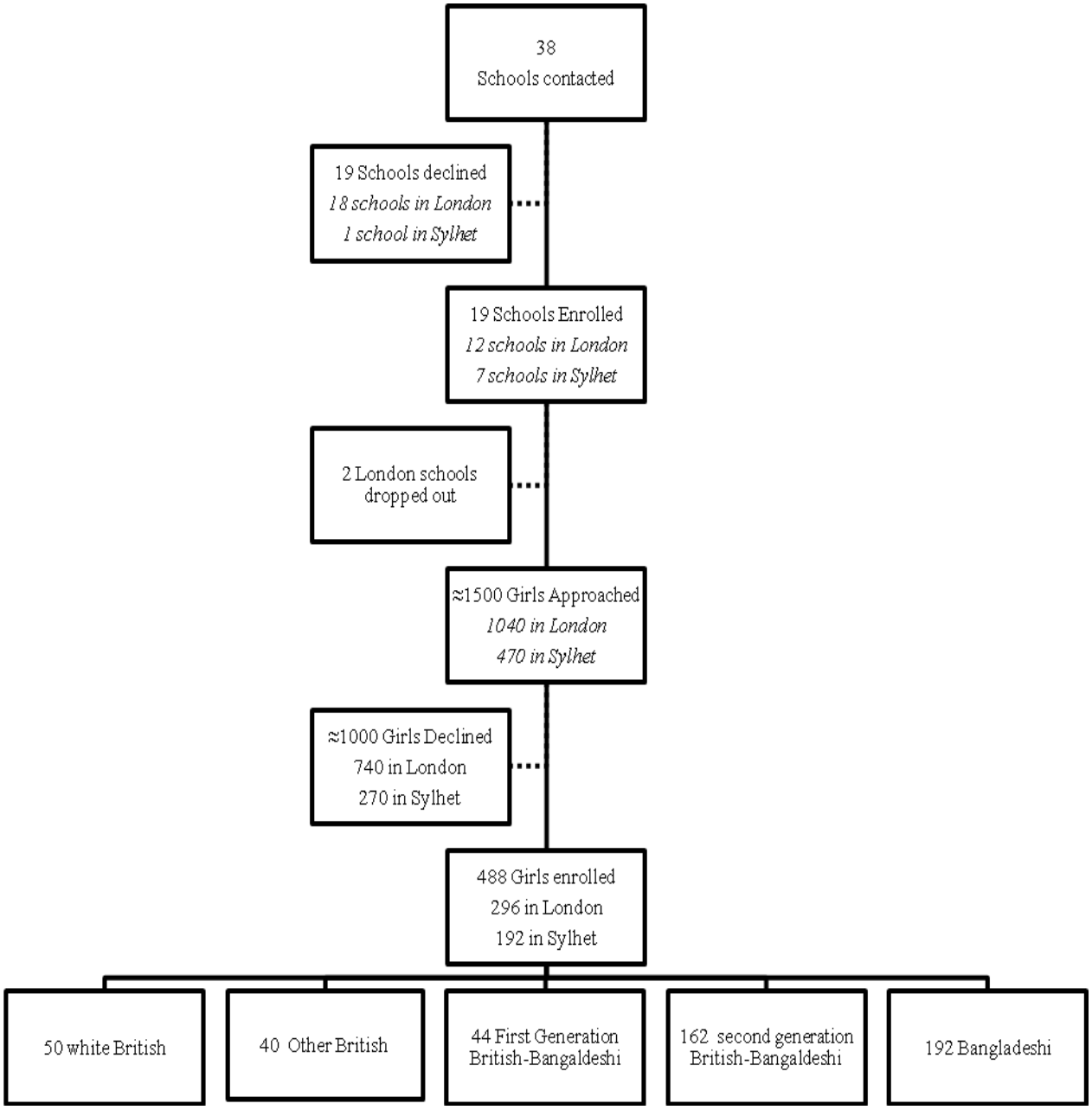
Participants were girls aged 5 – 16 years attending school between school years 1 – 10. Initial contact with participants occurred during school time, in female-only settings, where I explained the ABBY Project using photographic information sheets, samples of collection materials and an icebreaker activity. I gave the girls informational sheets and parental consent forms to be taken home. I visited the schools during the lunch hour to remind girls to return their signed consent forms. Before the interviews began the interviewer explained the project again and sought assent from the child.

Informational Sheets, Parental Consent and Child's Assent

A signed parental consent form had to be obtained in order for a participant to be officially enrolled into ABBY. When parental consent is obtained, children's assent is not legally required; nevertheless, it is good ethical practice to seek their views on whether they wish to take part in the study (NHS 2007). Two versions of an informational sheet were provided. One included a page of text relevant to ethics and the other included pictorial representations of the sampling methods (Appendix 1). The latter was the most effective in communicating the study for all age groups. It was especially helpful for Bangla-speaking and illiterate parents even though Bangla versions of the documents were available.

Once girls obtained a consent form signed by a guardian, they were enrolled in the study. The interviewer met with the participant, one-to-one, in a private room designated for ABBY data collection. The interviewer summarised the study to the participant using the pictorial informational sheet, showed the participant her guardian-signed consent and then asked her for her own assent pertaining to the various parts of the study. She was reminded that she could pass on any question and choose to stop her participation at any time. The participant determined for which parts she was willing to participate. In cases where a parent answered in the affirmative for specific parts, but at the time of interview the participant chose to decline, her preference was honoured. This did not apply if a parent answered in the negative. Once assent was confirmed, the interview commenced.

Figure 11: Flow Chart of School and Participant Recruitment



I conducted fieldwork commencing in September 2009 in London, England and completed in Sylhet, Bangladesh in April 2011 resulting in the enrolment of 17 schools and 488 girls into the ABBY Project using convenience sampling.

MOCK OF COLLECTION MATERIALS

In addition to explaining the pictorial informational sheets, it was useful to use mock collection devices when explaining how the biomarker specimens were to be collected. I showed the vials used to collect saliva. In addition to displaying the urine collection cup, I

showed them the opaque sealable bag that they would use to carry the urine sample to and from the toilets. This show-and-tell technique helped to reduce associated feelings of embarrassment when providing specimens.

Icebreaker Activity

To assist in participant recruitment, in some schools I facilitated an icebreaker activity during the informational session. The girls were each given a plastic cup containing normal water while one cup contained water that was dyed green. The girls were asked to walk around the room greeting other students, stating what was best about being a girl and exchanging water between their cups. After 10 minutes I asked the girls to raise their hand if their water had changed colour. I then asked how many girls had originally started with green water. When the girl who had received the green water raised her hand, it was illustrated that the green water that had started with only one person eventually had spread across the entire group. I summarized the activity by explaining that one person participating in the research could potentially help us to understand much more about people's health. This hands-on activity proved to be a fun and engaging way to promote participation in ABBY.

Retrieval of Consent Forms

Many girls were initially embarrassed when I described the ABBY Project and what it involved, but they showed enthusiasm and indicated that they were interested in participating. However, it was difficult to transform that enthusiasm into actual retrieval of a signed parental consent form. It was necessary to provide reminders to return their forms for girls of all ages. For primary school girls, I provided them with an embroiderer's thread bracelet in the colour of their choice to wear as a reminder to bring in their signed form. Later that week, I returned to the school at the lunch hour to collect signed forms and remind them to bring in the forms if they were still interested. For secondary school girls, the lunch hour drop-in was sufficient to remind the girls.

Participant Compensation

The participant received a growth chart with her height and weight measurements, a certificate of participation and a small gift of either earrings or a bracelet.

Sample Size

The timing of adrenarche assessed by DHEAS concentrations is the central, novel outcome of this study and so sample size calculations were considered for this variable. There are no existing data for salivary DHEAS levels among girls in the similar populations as those studied in ABBY. Therefore, during the design phase of the study, generic sample size estimation for ANOVA was derived. The estimated sample size (n=700) was determined using power analysis for ANOVA, a specified significance level (0.05), power (0.95) and medium effect size (G*Power, (Erdfelder et al. 1996). The group size of first generation girls was larger than the other groups because of the need to adjust for age at migration and time since migration.

After data collection, the UK sample contained fewer participants than planned, particularly due to difficulties in recruiting first generation British-Bangladeshi and white British girls. This is further explained in the Research Process section of this Chapter. On the other hand, recruitment in Bangladesh was easier and enabled more girls to be included in the study. Therefore, there were unequal sample sizes in each group.

A post-hoc power analysis was conducted using an exact significance test for inequality of proportions (age at adrenarche) between two independent groups (Sylheti and First Generation girls aged 5 to 7.5 years). This model achieved 70% power at the 0.04 significance level (Proportion p2 = 0.72; Odds Ratio = 0.23; N1= 45, N2 =11, allocation ratio N2/N1 = 0.24, α err probability = 0.05). This post-hoc power analysis does not account for double censoring which the parametric survival analysis model used to predict age at

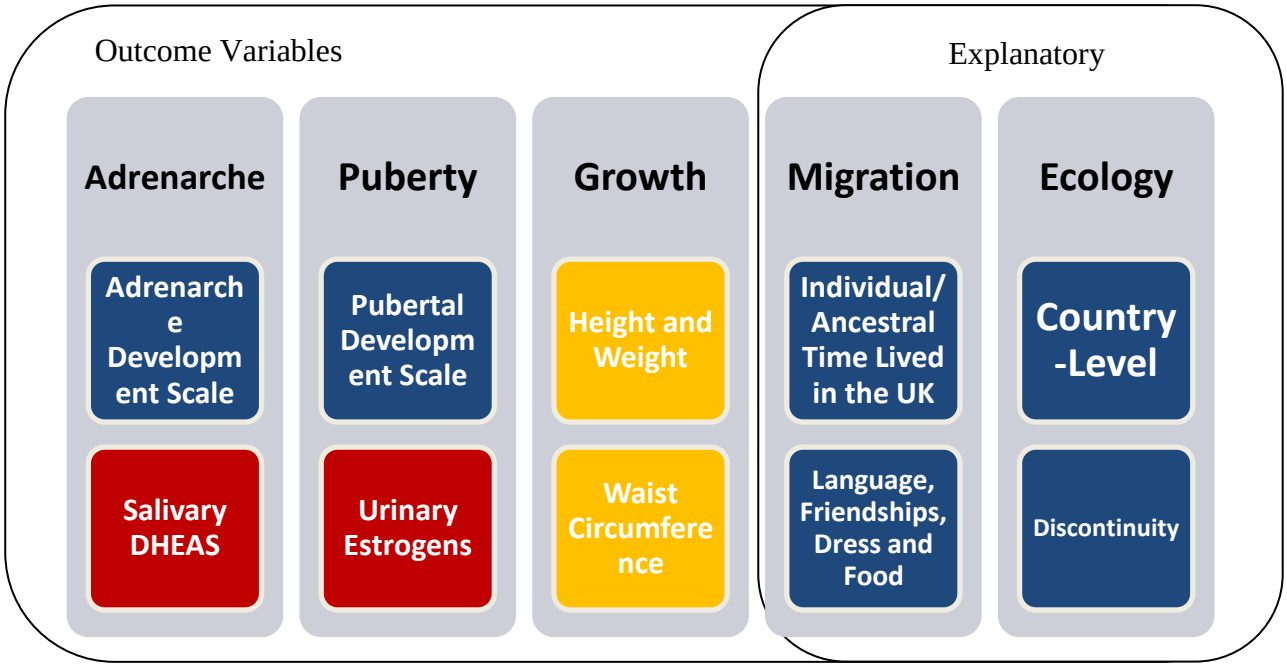
adrenarche in the final analyses of this thesis. Therefore, the actual power of the study to determine differences in the age at adrenarche may be below 70%. Also the first generation group was too small to make within group comparisons to either age at migration or time since migration. While the study was able to find statistically significant results pertaining to the first generation, interpretations of these findings need to take into account the small sample size. Replication studies with larger sample sizes may find smaller point estimates.

Phase 3: Data Collection

Research Instruments and Quantitative Data

A series of quantitative methods were used to collect information pertaining to Objective 1 (to assess whether there is variation in juvenile development by ethnicity, ecology and migration). These methods can be separated into three main categories: Questionnaire, Specimen Collection and Anthropometrics (Figure 12).

Figure 12: Schematic of the ABBY Project Variables and Methods



The methods are colour-coded so that questionnaire methods are in blue, specimen collection in red and anthropometrics in gold.

QUESTIONNAIRE

The study questionnaire was developed to assess variables related to physical development, behaviour and identity. In addition to these individual-level based questions, family demographics and socioeconomic measures were collected (Appendix 3). Each girl was interviewed one-on-one by a research assistant or me; 8 – 15 girls were interviewed per day. The interviewer asked each question to the participants and either checked the applicable answer or filled in the blank where necessary. There were at least two researchers in the room at all times and, at any time, two to five interviews were taking place (Photo 1).

Photo 1: Field assistants and participants completing questionnaires in an all-girl combined school in Sylhet Town, Bangladesh

PUBERTAL DEVELOPMENT SCALE AND ADRENARCHE DEVELOPMENT SCALE

Pubertal staging was assessed using the validated Pubertal Development Scale (PDS) which was developed by Peterson and colleagues as an alternative to Tanner staging (Petersen et al. 1988).

I adapted the PDS to incorporate an assessment of juvenile development. I also developed an Adrenarche Development Scale (ADS) which was incorporated into the PDS (collectively these will be referred to as the PDS-A, Appendix 4). This new scale included five additional

questions regarding secondary sex characteristics reported to be associated with adrenarche. These questions focused on lower leg hair, underarm hair, oily skin, spots or pimples and body odour. Following the format of the PDS, the answers to the adrenarche questions were scaled from 1 – 4 in progressing order. For some analyses the PDS-A were transformed into binary variables where 0 and 1 indicate the absence and presence of a physical characteristic, respectively. I modified the PDS question regarding pubic hair growth to ask about specific areas of the body including underarms, pubic area and lower legs.

The interviewer asked the participant whether she wanted to continue with the interview for the PDS-A or to complete this section herself. If she chose to self-report, this was recorded and the participant completed this section of the questionnaire in privacy. In Bangladesh, the PDS-A was shifted to later in the interview as the local field assistants suggested that it would be best to ask these more sensitive questions after they were able to establish a relationship of trust between themselves and the interviewee.

Twenty-four Hour Food Recall

To assess the type of foods that children eat, a 24-hour food recall was designed to ask about food consumption over the course of one day by asking about seven specific time periods. This included a mid-morning break during school time and a late night meal which is typically eaten by Bangladeshi families. This dietary recall included supplementary questions regarding how typical the last 24-hours were in food consumption and how often self-reported favourite foods were consumed. Because ABBY covered a wide age range of children and used food recall tools that have many limitations when working with children (Serdula et al. 2001), I chose a food recall method that would capture dietary quality (i.e., the types and patterns of food consumed) rather than dietary quantity (i.e., specific caloric and nutritional values).

IDENTITY

Markers of identity were measured in the questionnaire by asking about self-reported ethnicity, languages spoken at home and at school, preferences in dress and choice of friends. Asking about choices in dress and choices in friends in relation to ethnic groups has been used previously as a measure of acculturation with children living in east London (Bhui et al. 2005). In Bangladesh, we asked questions regarding language and religion to measure diversity within the Bangladeshi community. There were methodological issues with the ethnic terminology used in the questionnaire and so identity was explored more thoroughly using qualitative methods, as described under the section Qualitative Methods.

SALIVA AND URINE SPECIMENS

Saliva samples were collected to be measured for DHEAS. Saliva samples were collected in 5 ml polystyrene tubes using gum base (Cafosa ©, Barcelona, Spain) as a stimulant. Most girls produced at least 2 ml of saliva. Many of the youngest participants had difficulty in filling their tubes and so wider mouth cups were used for collection and the saliva was transferred immediately to collection tubes. Collection tubes were placed immediately in a cool box with ice until transported to the processing laboratory where the saliva was mixed using a vortex and then separated into two aliquots. The time of collection was recorded and all samples were collected between 09-16:00 hours. The samples were stored in -20º degree Celsius freezers until assayed for DHEAS. A total of 470 saliva samples were analysed at the Durham Ecology and Endocrinology Laboratory (DEEL) using ELISA assays purchased from Salimetrics©.

Urine samples were collected to be measured for oestrogens and oestrogens metabolites. The participant received a 100 ml-cup and discreet packaging to take to the designated study toilet. When she returned, the time was recorded on the cup and the sample was put directly on ice. All samples were collected between 09-16:00 hours. The urine was aliquotted and

stored in -20º C degree storage freezers until analysed for oestrogen and androgen metabolites. A total of 360 urine samples were analysed. Urinary oestrogens were analysed at the Laboratory of Proteomics and Analytical Technologies (LPAT) by a contractor of the National Cancer Institute, USA, by liquid chromatography- tandem mass spectrometry (LC-MS/MS) according to published methods (Xu et al. 2005).

Sample Processing, Storage and Re-collection

UK samples were transported on ice to a local hospital for processing and temporary storage until samples were later transferred to Durham University, 375 kilometres north of London. Bangladesh samples were transported on ice to Sylhet M.A.G Osmani Medical College (SOMC), where they were aliquotted and stored until shipped on dry ice to the UK. All UK and Bangladesh urine samples were shipped to a National Institutes of Health (NIH) biorepository in the USA until analysed. Unfortunately, in June 2010 the hospital freezers that stored the UK samples broke down twice and 300 samples defrosted. Because it was unknown during field work whether hormones were stable after more than one freeze/thaw cycle, some urine and saliva samples were re-collected. Girls (n=100) who had previously provided both urine and saliva were asked to provide a second round of samples. For this re-collection, a London research laboratory with alarmed freezers agreed to store all future samples until they were transferred to DEEL and thence to NIH. At the time of re-collection, the PDS-A and anthropometrics were re-collected. For the participants who provided additional samples, the re-collected samples were analysed in place of the previously stored sample. Samples were batched so that the effect of multiple freeze/thaw cycles could be considered in statistical analyses. The samples that experienced multiple freeze/thaw cycles will be referred to as freeze/thaw samples (FT) while the additional samples will be referred to as re-collected (RC). I also conducted a study to test the effect of repeated freeze/thaw

cycles on the target hormones in saliva (see details of this study under Phase 4: Method Validation).

ANTHROPOMETRICS

Anthropometric measurements, including height, weight and waist circumference were taken while the participant was clothed but without shoes and bulky outer clothing. In the UK, all anthropometry was conducted by only two researchers (LH and SB). In Bangladesh, all anthropometry was conducted by one of these researchers in order to maintain measurement consistency. Height was measured using a GPM Swiss anthropometer while weight was measured by a Tanita HD180 digital electronic scale (Tanita Corporation, Arlington Heights IL. USA).

Body mass index (BMI) was calculated by dividing weight in kilograms by stature in metres squared. Height-for-age, weight-for-age and BMI z-scores were calculated according to the method described by Cole et al. (1992) and compared to UK 1990 growth references (Cole et al. 1998) to determine growth status.

Because BMI does not distinguish between fat and fat-free mass, waist circumference measurements were used to assess abdominal fat (Lohman et al. 1988). Waist circumferences were measured with a flexible tape measure. The waist was measured to the nearest 0.5 cm with a tape measure, at the narrowest circumference between the chest and hips while the subject was in the standing position. Some girls in the UK (14%) who chose to participate in ABBY overall indicated that they did not want to be weighed or measured.

QUALITATIVE METHODS

The third phase of field work included participant observation and focus groups that I collectively refer to as qualitative data. I will start by describing participant observation, and then move to discuss the after-school clubs and focus groups.

PARTICIPANT OBSERVATION

Living in east London during field work allowed me to participate in certain aspects of community life. I attended various *Melas*: South Asian community celebrations with food, games, music and dance. I also attended events sponsored by Islamic Circles, a local Muslim, and predominantly Bangladeshi, community group. These lectures and meetings included Islamic history in Bangladesh, Youth Work with Young Asians, and Youth and Sexuality. I also volunteered at a local charity called London Archway that administered women and youth projects with the British-Bangladeshi populace in Tower Hamlets. These projects aimed to assist members of the local community in gaining training, work experience and eventual employment. I also volunteered for London Archway's youth programme by mentoring Girls' Evenings at their youth club. It was here that I developed relationships and interacted with 15 girls, both British and British-Bangladeshi, who came from severely deprived backgrounds. I also volunteered at a homework club with younger children. None of these girls participated in the interviews and sampling, but my time with them provided insight into the youth culture in east London. After attending these events, I recorded field notes at the end of the day.

In Bangladesh, I rented a home from a family I had met on a previous field visit. The house was part of a larger complex that belonged to seven brothers, sons of a prominent community member who owned hotels and had built the surrounding mosques. My rented accommodation was newly built by a family member who currently lived in the UK and owned a curry takeaway shop in a town near the seaside. He and his wife were building this home for rental until they retired. Living here, I was surrounded by the extended family and three sisters who were my hosts, friends and curry cooking instructors. They invited me into their home and to the weddings of seven family members. We spent many afternoons walking the neighbourhood or watching the sun set and the moon rise on their roof. My daily

interactions and conversations provided insight into family life in Sylhet and how middle-class young women spend their time.

In Bangladesh, fieldwork was possible due to the longstanding collaboration with Sylhet MAG Osmani Medical College (SOMC), a government teaching hospital located in Sylhet Town. SOMC provided office space, sample processing and storage facilities as well as personnel to assist in fieldwork efforts. Collaborating with SOMC provided many opportunities to participate in cultural events and celebrations. SOMC is a government medical college that instructs in English making communication with the community there possible. I attended the medical school's weekly scientific seminars and was invited to many of the college's cultural events including Independence Day, National Language day and a Hindu festival. I was also invited to departmental luncheons and picnics. My experiences here provided insight into biomedical training, public health initiatives and governmental health institutions in Bangladesh.

AFTER-SCHOOL CLUBS

I conducted most participant observations primarily through facilitating after-school clubs.

I organised three clubs in the UK:

1. Girls Health and Fitness Club: six weeks with Year 4 girls, aged 7 – 8 years, n=8
2. GAL (Girls at Lagerfield) Club: six weeks with Year 6 girls, aged 11 – 12 years, n=22
3. Fit-4-Life Club: 36 weeks with Year 10 girls, aged 14 – 15 years, n=20

The after-school clubs provided me with opportunities to explore the experience of growing up by both taking part in informal discussions and by facilitating focus groups. For the

duration of the after-school clubs, the organisation and style of the sessions shifted. The sessions began as very structured; but, as we became more familiar with each other, the girls began to direct how and what kinds of activities we engaged in. Overall, the most fruitful interactions would occur when the girls knew I would be in the classroom and they would visit to chat. During the sessions, I recorded mental and written notes and after the sessions I recorded full field notes (Lofland 2006).

GIRLS HEALTH AND FITNESS CLUB AND PRIMARY SCHOOL 1

This six week club included eight girls aged 7 – 8 years who met with me once a week. Each session was organised to include a healthy cooking session, a physical activity and a structured activity that was documented by worksheets or field notes. The structured activities included: writing poems about being a girl, listing foods they eat, creating family trees and measuring each other's height and weight.

GAL CLUB AT PRIMARY SCHOOL 2

The GAL Club was offered to a partnership of schools, comprised of one secondary school (SS1) and its feeder schools who had agreed to participate in the ABBY Project. After discussions with the partnership coordinator, who identified the transition between primary and secondary schools as a sensitive time, we decided to offer an after-school club for Year 6 girls, aged 11 – 12 years. The after-school club would be an innovative way of exploring issues specific to this age group as well as providing a service for the schools. The ABBY Project supported Year 6 girls by offering a transition club that included hands-on and engaging exercises in a girls-only environment. This two-fold approach to research was conducted with the hope of guiding future transition clubs for Year 6 girls.

I visited the primary schools in the first half of the summer term to tell girls about the GAL Club and to distribute permission slips. These permission slips asked for the parents' permission for their child to participate, for photos to be taken of their child during the clubs' activities and for observations made to be used to supplement the ABBY Project research while assuring confidentiality and anonymity.

The GAL Club took place on Wednesday afternoons over a six week period during the second half of summer term. All Year 6 girls from three primary schools were invited to attend the club and 24 girls initially signed up. During the six weeks, the girls engaged in a

range of activities all addressing the topic of happy, healthy lives. The GAL Club used visitors to run three of the sessions and this helped to introduce expertise from different areas including: life-coaching, photography and cooking. I observed the sessions and kept field notes of club events. A list of activities that took place can be found in Appendix 5.

FIT-4-LIFE AT SECONDARY SCHOOL 2

The objective of the Fit-4-Life Club was to provide a forum where girls could learn and discuss ways of leading happy, healthy lifestyles. Through novel, interactive and culturally sensitive activities, girls were encouraged to explore their short and long term aspirations and understand how science may be a pathway to achieving their goals. Many aspects of ABBY overlapped with the National Science Curriculum and these connections were highlighted during the after school club in hands-on and engaging exercises.

Over the course of the 36 weeks with the Fit-4-Life Club, the organisation and style of our sessions shifted. I spent the most time with UK Secondary School 2 (SS2) through informal sessions, structured club sessions and community events related to this school. With the financial assistance of the borough, Olympics and the UK Lottery, this particular school was in a transitional period from having one of the worse reputations for violence and underachievement to becoming a magnet school for science and technology. It was part of a larger community regeneration effort supported by a local member of parliament. Meeting school faculty at this time of transition allowed for ABBY to join in with these efforts by promoting science education in ways that were relevant to the local community. In addition to organising an after-school club, I also attended monthly community coffee meetings, participated in a community Summer Fun Day and organized a community discussion group with students and community members about healthy food and food growing. I also spoke to all Year 11 students in assembly about pursuing higher education. I became a regular volunteer at the school and this led to building trusting relationships with teachers and

students at the school as well as surrounding community members. A list of activities that took place over the course of the Fit-4-Life club can be found in Appendix 5.

Focus Groups

By facilitating after-school clubs in the UK, certain themes emerged around dress, food and definitions of growing up. I organised three focus groups to explore these themes with Fit4Life club members (n=12). I held two additional focus groups with parents to discuss growing up (n=10). I sought comparative data in Bangladesh by organising three focus groups led by myself or field assistants around the themes of food and clothes. In Bangladesh, time constraints and language barriers made it difficult to facilitate after-school clubs. Alternatively, I organised focus groups either as part of supplementary science classes that I taught or during existing school activities. Organising focus groups led by field assistants allowed for the themes of identity, food and clothes (that had emerged in the UK) to be explored in Bangladesh.

Field notes

Over the course of my time in east London and Sylhet, I kept a field journal and I refer to this journal when I recall an observation or discussion. I coded the observations using key themes that were identified. Many of the focus group members had also participated in the quantitative part of ABBY. For some factors, such as diet and identity, I was able to triangulate questionnaire data with my observations and discussions by linking such qualitative and quantitative data.

PHASE 4: ANALYSES AND METHOD VALIDATION

All relevant statistical and coding analyses are described in Chapters 3, 4 and 5. The following experiments and analyses were performed as part of the data preparation step to validate that the methods used were accurately measuring the desired variables and to determine how best to proceed with analyses. These method validation studies were performed for salivary DHEAS, urinary oestrogens and the Adrenarche/Pubertal Development Scale (PDS-A).

SALIVARY DHEAS

I assessed three potential complications for the use of salivary DHEAS as a marker of adrenal androgen production among girls aged 5-16 years:

- Do DHEAS levels differ by the time of collection?
- Do DHEAS levels differ by number of freeze/thaw cycles?
- Are salivary DHEAS levels sensitive and reproducible among juvenile and adolescent girls?

Time of Collection: There was a small but significant relationship between salivary log of DHEAS levels and the time of day at which the sample was collected (Figure 13). Multiple linear regressions were used to quantify the association of time of collection with the log of DHEAS levels, after adjusting for age. The total variance in DHEAS explained by time of day was 2.7% and was statistically significant ($\beta = 0.12$; $p=0.003$). Although this is a small effect, to be safe, time of collection was entered into the models as a covariate and reported if it changed the associations.

Multiple Freeze/Thaw Cycles: Mean concentrations of DHEAS were compared based on whether the sample had been defrosted more than once. The mean concentrations and

standard deviations of DHEAS (log) for FT and RC samples were 6.8 +/- 1.3 and 6.7 +/- 1.4 pg/ml, respectively (Figure 14). Multiple linear regressions were also used to assess the association of freeze/thaw cycles with DHEAS levels, after controlling for the influence of age of participant and time of collection. There was no association of multiple freeze/thaw cycles with DHEAS levels (β = -0.14 +/- 0.12; p=0.26).

Sensitivity and Reproducibility: DHEAS was analysed utilizing 15 batches over the course of two months. Samples were evenly distributed by age and migration group across the plates and then randomly ordered on each plate. A pooled quality control sample was included on each plate. Samples were run in duplicates and any samples with batch coefficients of variation (CV) higher than 22% for DHEAS were analysed a second time. Overall CVs were less than 20% and the lower and upper limits of detection were 43 pg/ml and 16,000 pg/ml respectively. DHEAS was detectable in all samples. Twelve samples had DHEAS concentrations too low to be quantifiable, all of which were from girls aged less than eight years, suggesting that the low levels belonged to girls who had not reached adrenarche. An additional 12 samples were analysed at a one to two dilution due to having an insufficient amount of specimen. The concentration was multiplied by two to be comparable with the other samples. According to the assay kit manufacturer, this dilution results in +/- 20% recovery rate (Salimetrics ©).

Figure 13: Scatter Plot and Lowess Smoother of the Log of DHEAS and Time of Collection

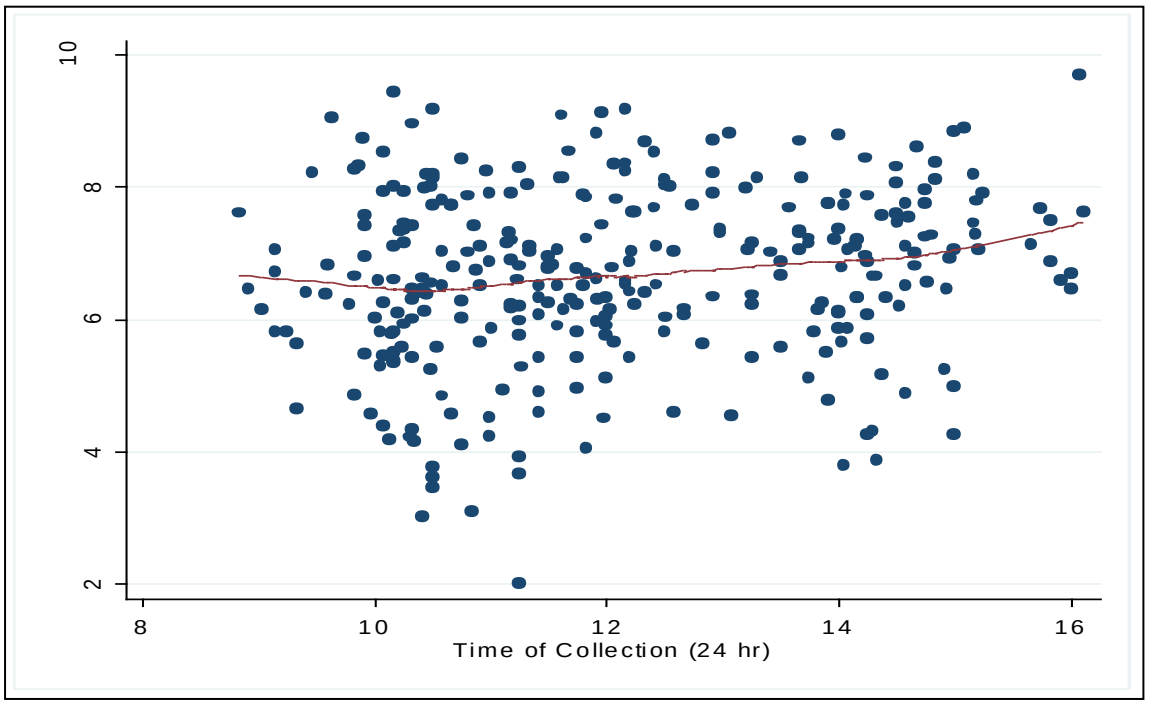
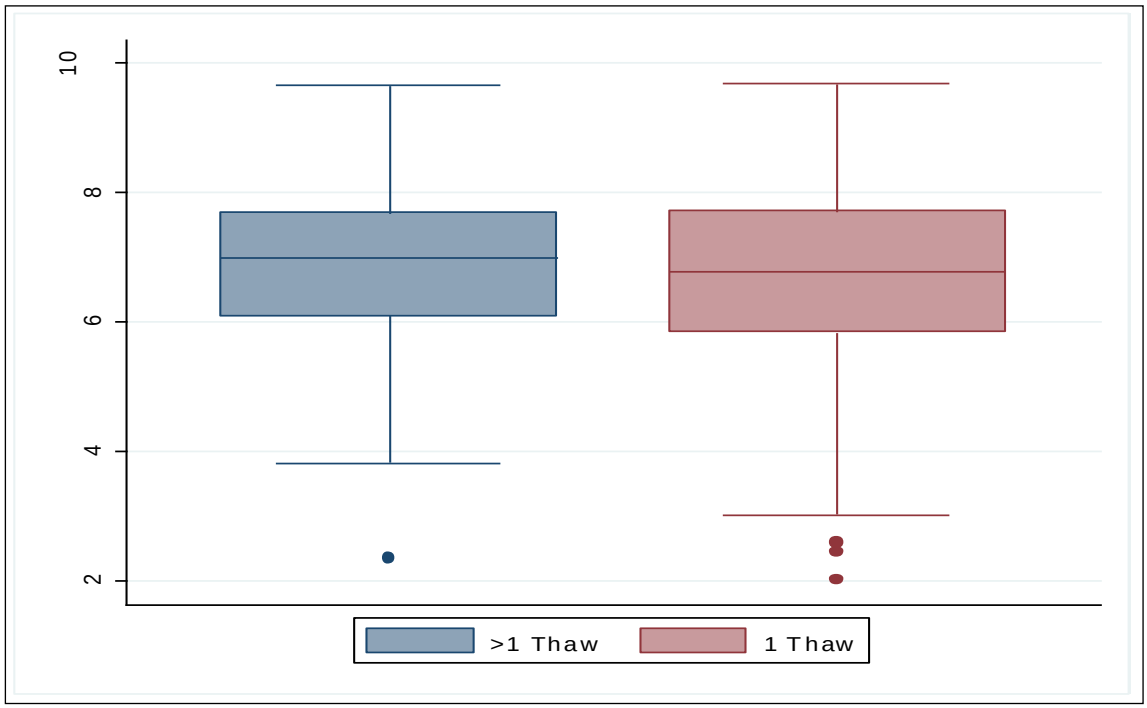


Figure 14: Mean Levels of DHEAS (Log) According to Freeze/Thaw Cycles



URINARY OESTROGENS

I assessed three potential complications for the use of the sum of urinary oestrogen/oestrogen metabolites (oestrogen) as a marker of oestrogen production among girls aged 5 – 16 years:

- Do oestrogen levels differ by the time of collection?
- Do oestrogen levels differ by number of freeze/thaw cycles?
- Are oestrogen levels sensitive and reproducible among juvenile and adolescent girls?

Time of Collection: After adjusting for age, multiple linear regressions were used to assess the time of collection on the log of oestrogen levels. There was no significant relationship between oestrogen levels and the time of day at which the sample was collected ($p = 0.401$) (Figure 15).

Multiple Freeze/Thaw Cycles: Mean concentrations of oestrogen were compared based on whether the sample had been defrosted more than once. The mean concentrations and

standard deviation of oestrogen (log) for FT and RC samples were 2.6 +/-1.4 and 2.6 +/-1.1 pmol/mg creatinine, respectively (Figure 16). Multiple linear regressions were also used to assess the effect of freeze/thaw cycles on oestrogen levels, after controlling for the influence of age of participant. There was no effect of multiple freeze/thaw cycles on the oestrogen levels (β = -0.12 +/- 0.12; p=0.321).

Sensitivity and Reproducibility: A total of 400 urine samples were analysed the US National Cancer Institute at the Laboratory of Proteomics and Analytical Technologies (LPAT) using liquid chromatography-mass spectrometry (LC-MS/MS) according to previously published methods (Xu et al. 2005). Fifteen oestrogens and oestrogen metabolites (oestrone, oestradiol, oestriol, 16-epiestriol, 17-epiestriol, 16-ketoestradiol, 16α-hydroxyestrone, 2-methoxyestrone, 4-methoxyestrone, 2-hydroxyestrone-3-methyl ether, 2-methoxyestradiol, 4-methoxyestradiol, 2-hydroxyestrone, 4-hydroxyestrone, and 2-hydroxyestradiol) collectively referred to as oestrogen were measured in one array. Quality control (QC) samples were included (n=40), each composed of one of four pooled samples, to determine within- and between- batch coefficients of variation (CVs). The overall CVs (sum of within- and between- batch CVs) for replicate quality control samples (averaged for different concentrations of steroids) were below 5%. All samples were detectable and above the limit of detection. Although a fraction of samples were below the assay's published limit of quantitation (4 pg/0.1ml), the low CVs indicate that the assay was able to quantify levels below 2.5 pg/0.1ml. Even among the least abundant oestrogen (4-methoxyestradiol , 2-methoxyestradiol, 4-methoxyestrone, 2-hydroxyestrone-3-methyl, 4-hydroxyestrone), CVs ranged from 3-5% for concentrations as low as 0.72, 2.37, 2.95, 3.28, 3.43 pg/0.1ml. Fifteen oestrogens were measured in one array, and concentrations were received from the laboratory in pg/0.1ml. Aliquots from the same parent samples and QCs were also analysed for creatinine by PPD © using an enzymatic colorimetric assay; all CVs were below 2%.

Oestrogen concentrations were adjusted for creatinine and the units were converted to pmol/mg of creatinine. All oestrogens were summed to create a total measure of oestrogen

Figure 15: Scatter Plot of Total Oestrogen and Time of Collection

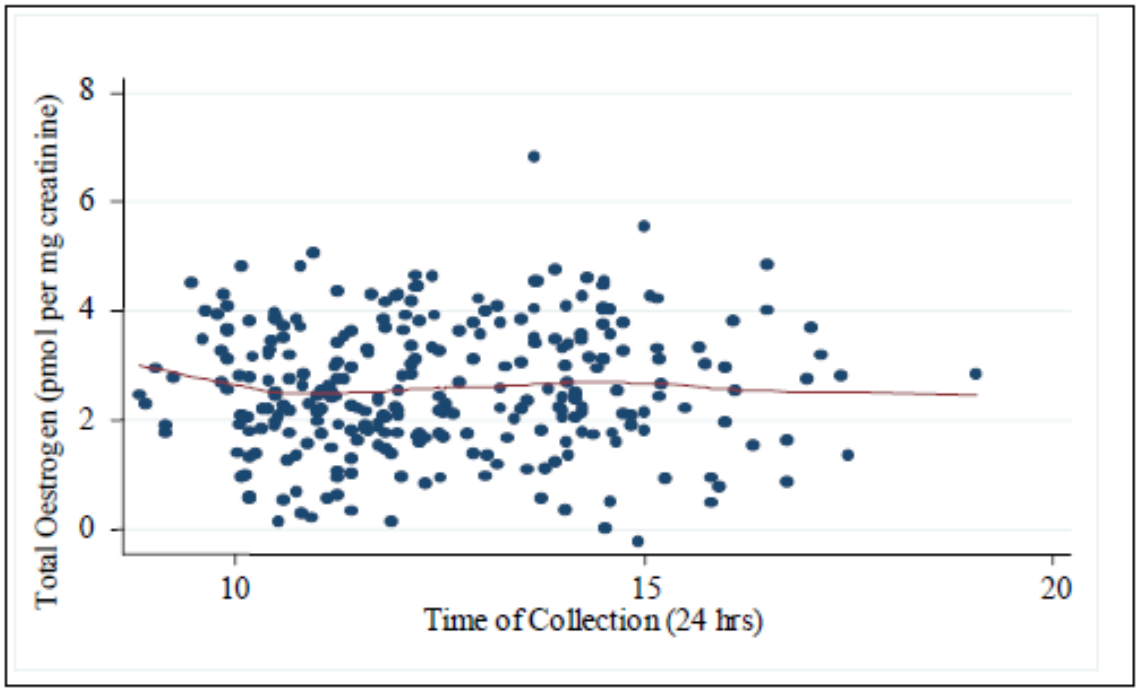
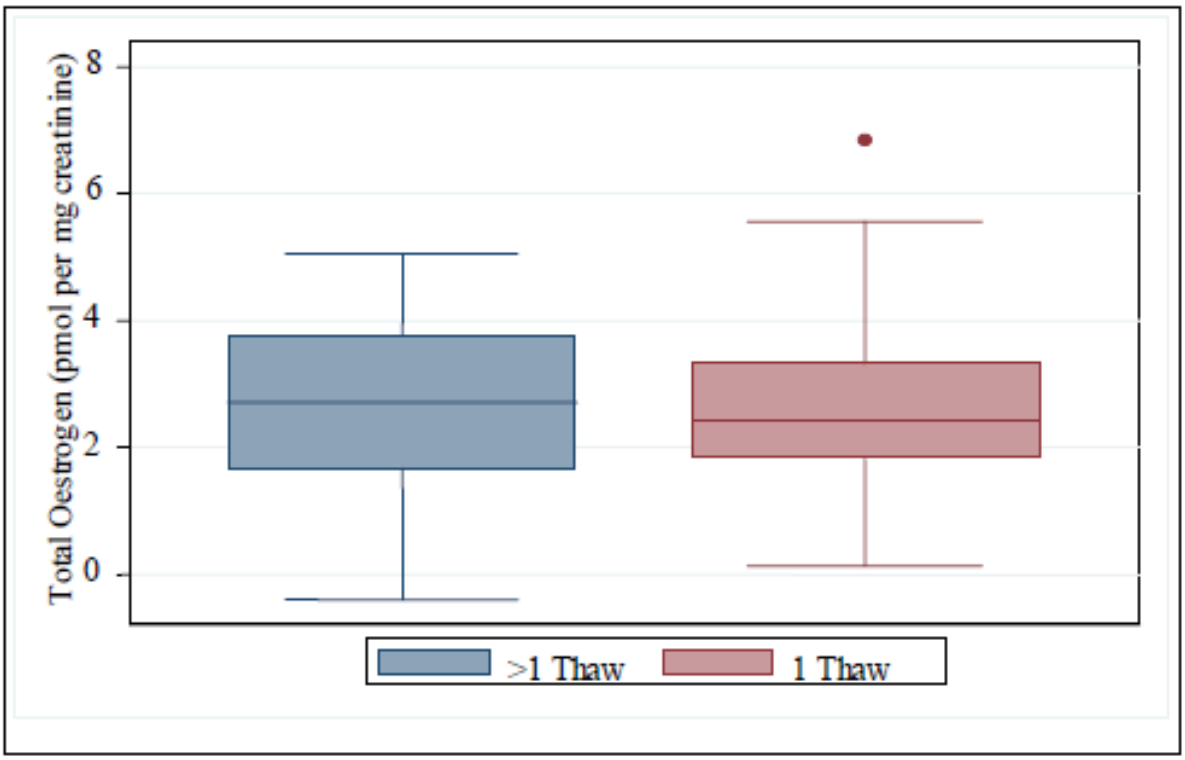


Figure 16: Mean levels of Total Oestrogen by Freeze/thaw cycles



ADRENARCHE/PUBERTAL DEVELOPMENT SCALE

The Adrenarche/Pubertal Development Scale was validated by comparing self-reported presence of juvenile and pubertal secondary sex characteristics with salivary DHEAS and urinary oestrogen levels. Because androgens and oestrogens may mediate the presence of some secondary sex characteristics, the association between self-reported sex characteristics and each hormone marker were compared in two main ways:

1. Descriptively:
    a. Are DHEAS levels higher among girls who report juvenile secondary sex characteristics than those that do not?
    b. Do levels of DHEAS and oestrogen increase with increasing breast and pubic hair stage?

c. Are levels of oestrogen higher among girls who have reached menarche compared to those who have not?

2. Quantitatively:
    a. Logistic regressions were run testing for differences in DHEAS and oestrogen by presence or absence of each secondary sex characteristic, stratified by migration group.
    b. Linear regression was used to test if levels of DHEAS and oestrogen increased with development of breast and pubic hair. The models were also adjusted for migration group.

DHEAS increased with the presence of each juvenile and pubertal secondary sex characteristic and menarche (Figures 17-19). As DHEAS increased (by a factor of three), the unadjusted odds of having different juvenile secondary sex characteristics also increased (Odd Ratios: Underarm Hair = 1.98, $p<0.0001$; Lower Leg Hair = 1.50, $p<0.0001$; Pimples = 1.64, $p<0.0001$; Oily Skin = 1.33, $p<0.0001$; Body Odour = 1.33, $p<0.0001$).

DHEAS increased with each stage of breast development ($\beta= 0.74$ $p<0.0001$) and pubic hair growth ($\beta= 0.42$ $p<0.0001$). DHEAS was also higher among girls that had reached menarche ($\beta= 1.25$; $p<0.0001$). The association did not change when the models were adjusted for migration scale.

Oestrogen increased with each stage of breast development ($\beta= 0.54$ $p<0.0001$) and pubic hair growth ($\beta= 0.50$ $p<0.0001$) (Figures 20-21). Oestrogen was also higher among girls that had reached menarche ($\beta= 1.6$; $p<0.0001$) (Figure 22). The association did not change when the models were adjusted for migration scale.

Figure 17: Log of DHEAS (pg/ml) Levels Among Girls With and Without Juvenile Secondary Sex Characteristics; (A) Axillary Hair (B) Leg Hair (C) Pimples (D) Oily Skin (E) Body Odour.

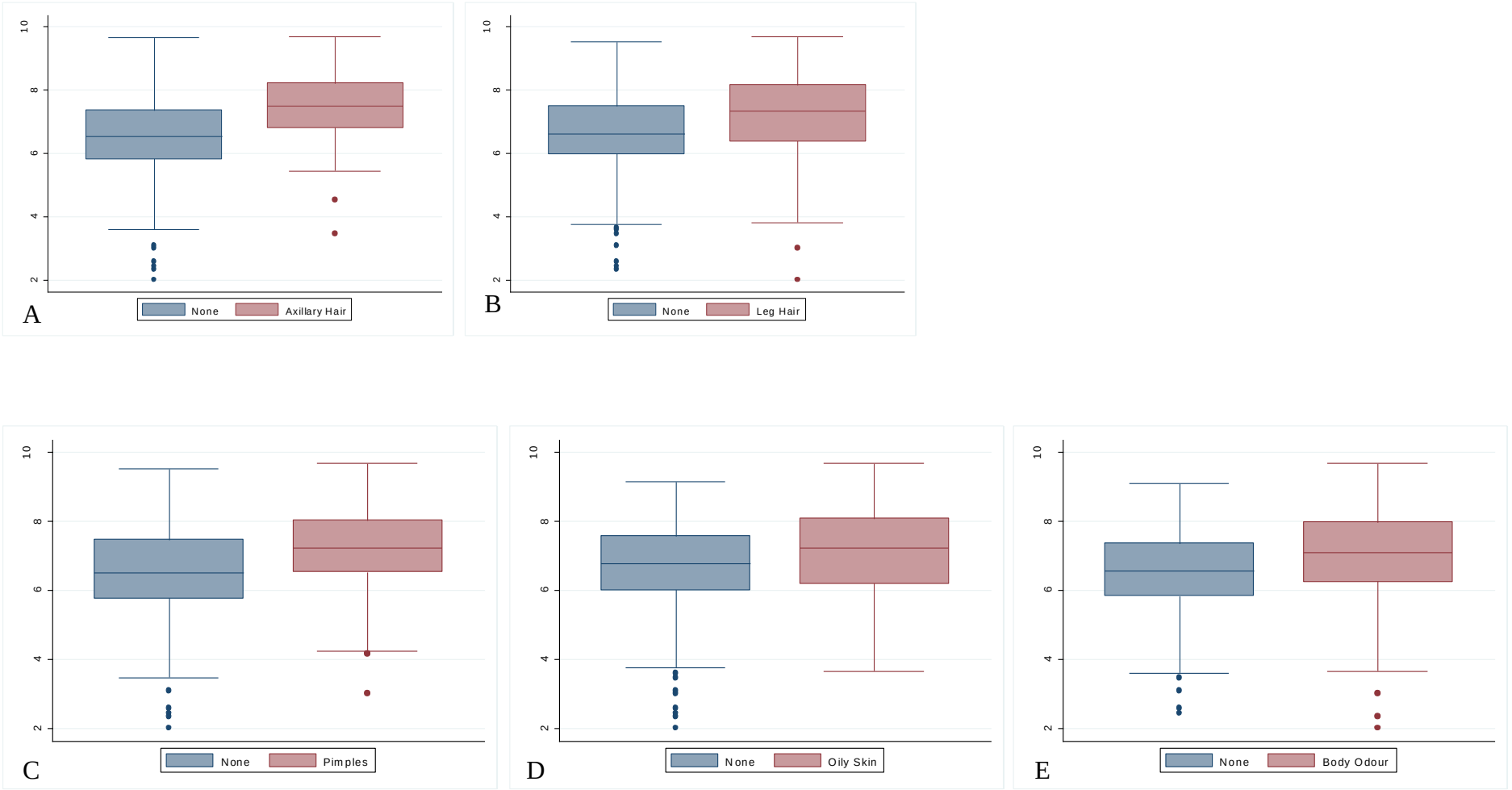


Figure 18: Comparison of Log of DHEAS According to Breast Stage Among Migration Groups

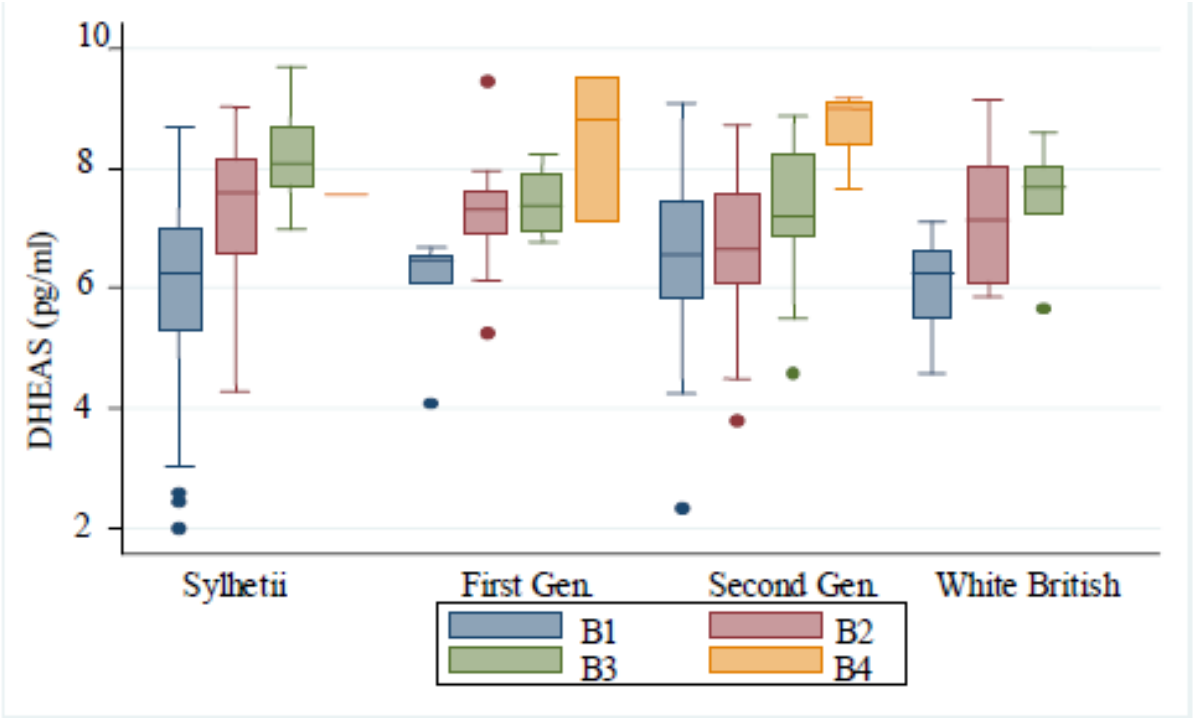


Figure 19: Comparison of Log of DHEAS According to Pubic Hair Stage Among Migration Groups

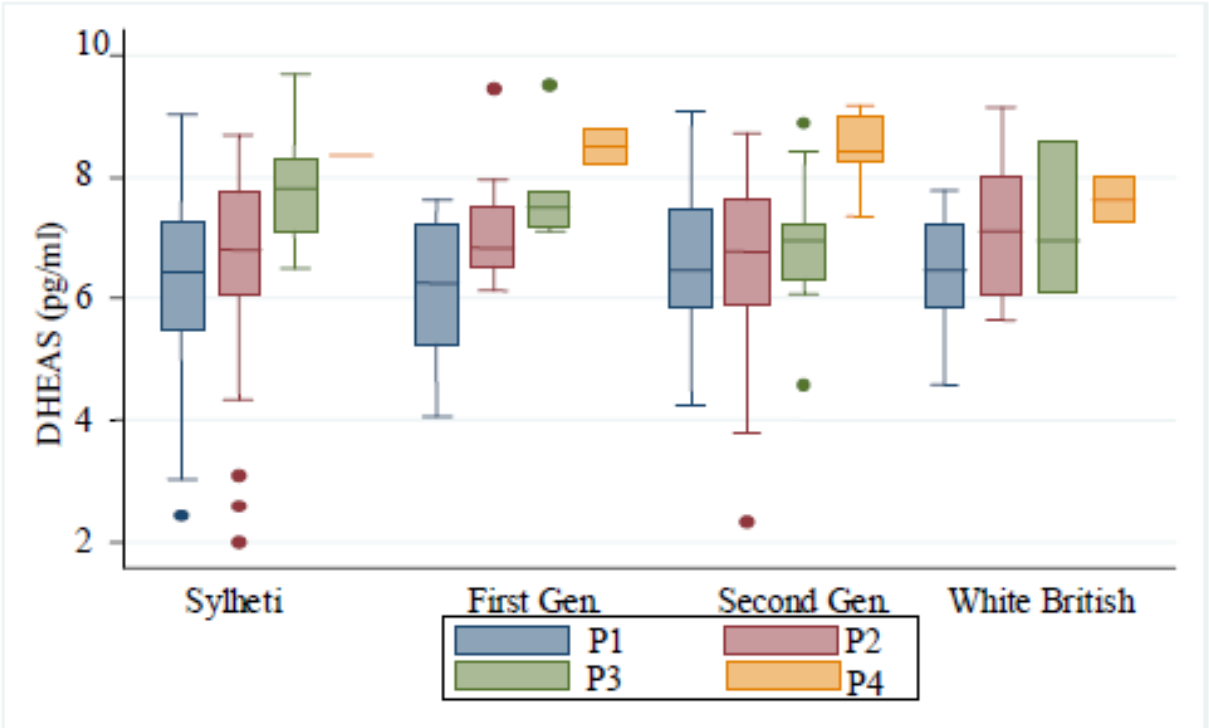


Figure 20: Comparison of Log of Total Oestrogen According to Breast Stage Among Migration Groups

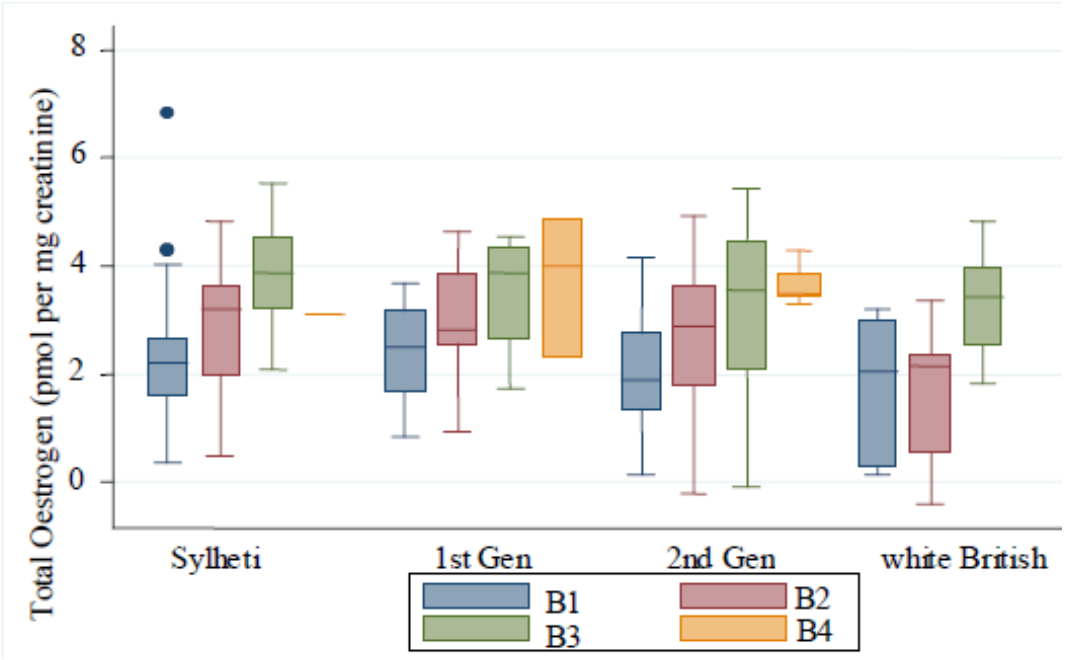


Figure 21: Comparison of Log of Total Oestrogen According to Pubic Hair Stage Among Migration Groups

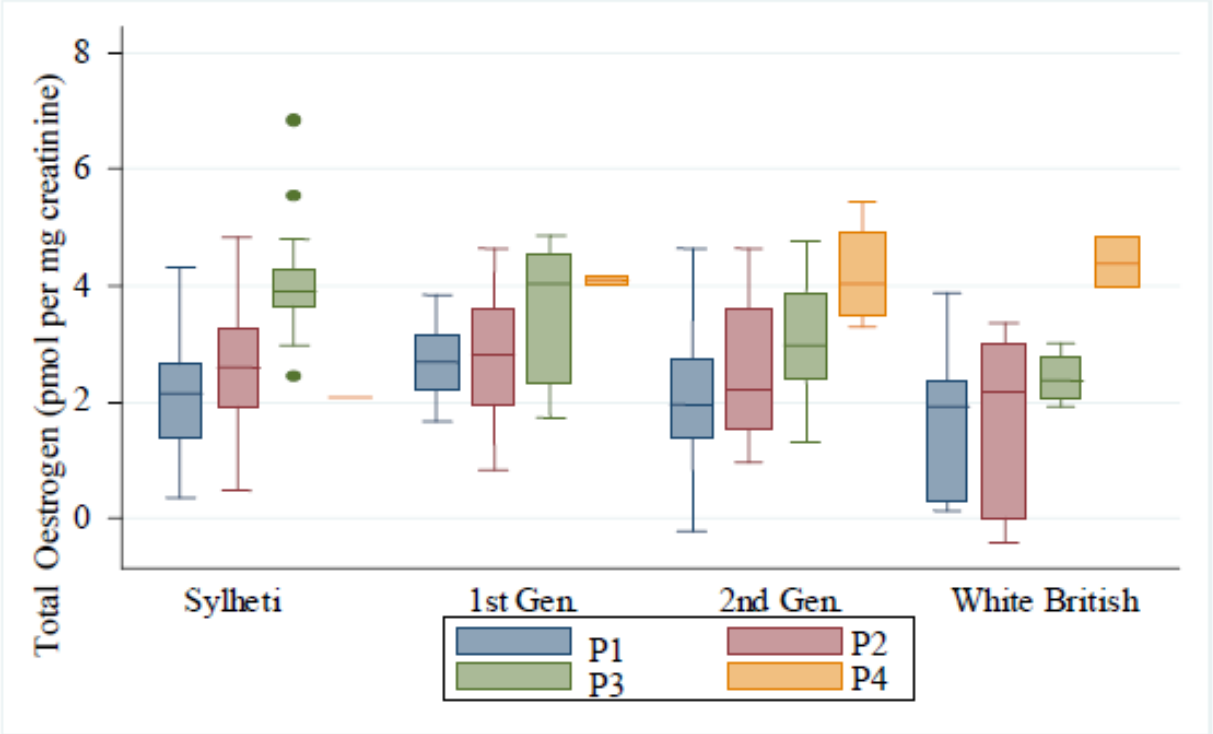
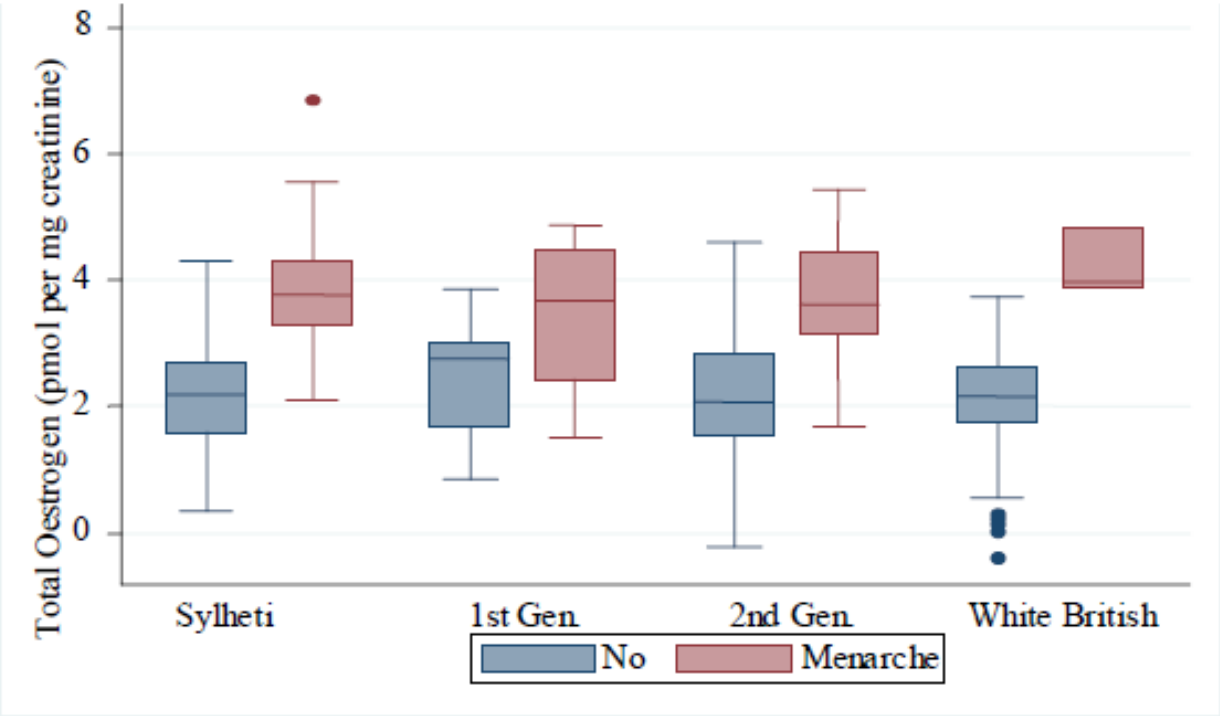


Figure 22: Comparison of Total Oestrogen by Menarche Status and Migration Group



The Research Process

Ethical Approvals

The ABBY Project received ethics permission from the Department of Anthropology Durham University Ethics Committee and the Sylhet MAG Osmani Medical College. An Institutional Review Board (IRB) exemption from the Office of Human Research Subjects at the National Cancer Institute was issued based on prior ethical permission from Durham University and SOMC. A material transfer agreement (MTA) was put in place between NCI, SOMC and Durham University. The combination of these ethics approvals and exemptions fulfilled legal requirements to conduct research. During the UK-based research, Tower Hamlets Local Authority inquired about the research and required that their local ethics board review the study. While this is not a legal requirement, I submitted an application which was

approved. All researchers and assistants received Criminal Records Bureau (CRB) clearance within the UK.

Participant Privacy was ensured. All participants were granted an anonymous identification number. Unidentified data were input into the project's database, which was stored on password-protected computers. The coded list was kept separately in a locked drawer. Pseudonyms individuals are used throughout this thesis.

THE RESEARCH TEAM

The research team consisted of the following individuals:

- Field coordinator (female, late 20s, American/Anglo-Indian): I led the recruitment, data collection, after-school clubs and focus groups. I processed all specimens in the UK. I supervised all fieldwork activities in both field sites
- Research assistant (female, early 30s, Sylheti/Bangladeshi): assisted in interviews, sample collection and interpreted during recruitment in London
- Research assistant apprentice (female, late 30s, Sylheti/Bangladeshi): assisted in recruiting first generation British-Bangladeshi girls in London
- Five field assistants (female, early 20s, Sylheti/Bangladeshi): recruited schools and conducted all interviews in Sylhet
- Laboratory assistant (male, late 30s, American): processed biological samples in Bangladesh.

RESEARCH ISSUES IN THE UK

Recruiting first generation British-Bangladeshi girls was difficult and required a targeted approach. Therefore, a research assistant apprentice (RAA) was hired and was responsible for identifying first generation British-Bangladeshi girls living in Tower Hamlets. The RAA was

a Bangladeshi woman with school-aged children and links within the Bangladeshi community in her area. Despite her background, she found that many mothers distrusted her inquiry and they expressed anxiety as to the purpose of the research. Some of the reasons for their distrust may be a result of issues surrounding a family's immigration status and a perceived threat of deportation.

Another school reported having 100 girls who were born in Bangladesh and initially agreed to take part in ABBY. The school later decided to no longer participate because I had asked specifically to work with their first generation students and the school felt this could lead to potential problems by singling out these students. Despite targeted efforts, the stigmas attached to being a first generation British-Bangladeshi girl (explained further in Chapter 5) made it difficult to recruit more girls from this population into the study.

Recruiting white British girls was also difficult as the majority of students in the participating schools were Bangladeshi. It was important to recruit girls living in similar neighbourhoods, attending the same school to account for potential socioeconomic, environmental and other confounding factors. In order to recruit more white British girls, recruitment would have needed to be extended to other parts of London where fewer Bangladeshi families lived. Therefore, the white British migration group is relatively small compared to the other groups.

LANGUAGE

Language: Informational sheets, consent forms and the questionnaires were available in English and Bangla. These were translated by a Bangladeshi researcher and then back-translated to English by another Bangladeshi researcher in order to check for consistency. All focus groups were conducted in English except when assistants in Bangladesh facilitated the groups. All field assistants spoke Bangla, English and Sylheti, the local Bangladeshi dialect. In autumn 2010, I enrolled in Bangla for Beginners, a language course provided by

Tower Hamlets Community Education Centre. I was introduced to the Bengali script and learned the basics of conversational Bengali including greetings, colours, foods, kinship terms, numbers, etc. This class was very helpful when I went to Sylhet as I was equipped with basic communication skills that helped when meeting and greeting key contacts and participants.

COMMUNICATING IN THE FIELD

Once I was in the field, communicating the research effectively to participants became a challenge. Linking ABBY to child development was too vague for potential participants and their guardians. Yet explaining the evolutionary significance of adrenarche was too detailed. Finding a communication gap between scientists and the general population is no surprise. The Children Environmental Health study (Israel et al. 2005) has used community-based participatory research and reports that differences in languages, complex scientific wordiness versus colloquialisms and quick email versus verbal communication all present challenges when conducting this type of research. Communicating across this gap in an effort to conduct scientific research with the general public became necessary if ABBY was to meet its objectives; though it was not as easy to execute the objectives as it was to acknowledge these issues. The challenge was to contextualise ABBY within a broader public health issue that generated interested without providing false promises or hopes. I was able to make ABBY viable by highlighting its connection with breast cancer research. While breast cancer is not an immediate endpoint of the study, the study of hormone variation is implicated in understanding the aetiology of breast cancer.

RESEARCH ISSUES IN BANGLADESH

In Bangladesh, ABBY liaised with Shajalal University in Sylhet Town to hire five field assistants from the Sociology, Anthropology and English departments. A specific ABBY

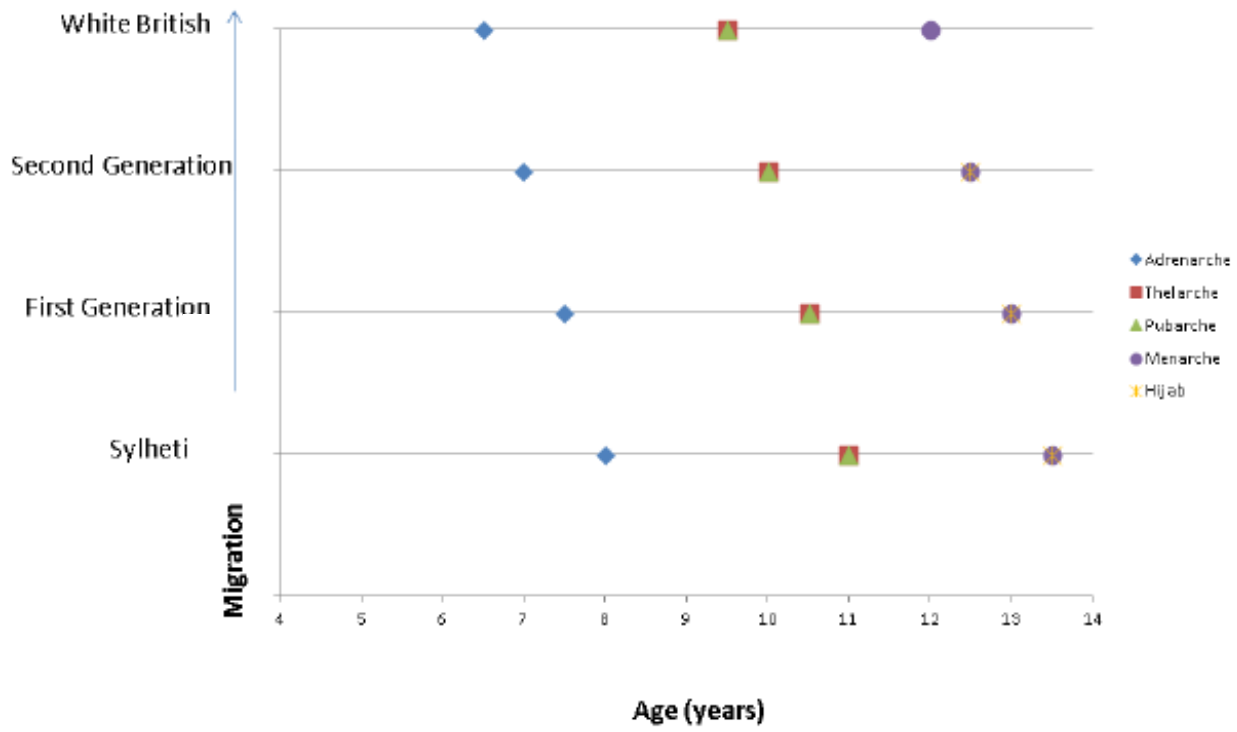
Project training was developed to explain the rationale behind conducting such research and to train the research assistants in the methodologies used. Training included sample collection, interviewing and anthropometry. The field assistants initially expressed their own hesitation with asking sensitive questions especially during the pubertal development questionnaire, so field training focused on interview skills, body language and communication style to help the research assistants overcome their own hesitations.

The field assistants provided additional insight into young Sylheti women's lives. They spoke of their own experiences while collecting data and offered their opinions on my observations after data collection sessions. All field assistants signed consent forms and agreed to be part of the research.

CONCLUSION

In summary, the ABBY Project used a wide array of quantitative and qualitative methods selected before, and developed during, the fieldwork process. Data were collected through participant observation in two countries, interviews with over 500 girls and collection of specimens from 488 individuals. ABBY compared juvenility across populations and followed the biocultural model, using mixed methods. The next three Chapters use these methods to explore the timing of juvenility and puberty and the experience of growing up among Bangladeshi and British girls.

Figure 23: Hypothesised Timing of Juvenile and Pubertal Development Among ABBY Migration Groups



The overall hypothesis for ABBY is that the timing of adrenarche, thelarche, pubarche and menarche will occur earlier with increasing individual/ancestral generations lived in the UK. Hijab may be a cultural marker of pubertal development among Bangladeshi girls.

CHAPTER 3: THE TIMING OF ADRENARCHE AMONG BANGLADESHI AND BRITISH GIRLS

SUMMARY

In this Chapter, I investigate the timing of adrenarche across populations with different early life environments. The developmental environment in Bangladesh differs from the UK due to economic differences that are manifested in the key areas of access to healthcare and sanitation services. When Bangladeshi women migrate to the UK there are changes in their health. For example, their salivary progesterone levels increase substantially, particularly if they were children when they immigrated to the UK. The lifestyle changes accompanying migration have not been identified, but exposures that occur in childhood and early adolescence may influence subsequent health, such as reproductive function. Adrenarche, specifically, is perceived as a biological event that is susceptible to such exposures, but international differences in the timing of adrenarche have not yet been established. This Chapter investigates the pattern of adrenarche development among Bangladeshi, Bangladeshi migrant groups and white British girls.

Before presenting those data, I define adrenarche for this study's purposes. Next, I review the biology of adrenarche, explain possible reasons driving its evolution and discuss whether the timing of adrenarche varies within and between populations. Finally, anthropometrics during juvenility, concentrations of DHEAS and the presence of juvenile secondary sex characteristics are analysed in an effort to compare the size and timing of adrenarche across populations of Bangladeshi and British girls.

INTRODUCTION

Adrenarche is an early life event that marks the beginning of juvenility. Previous researchers have highlighted the juvenile period as an important stage in the human life course (Bogin 2001,Campbell 2006, Hochberg 2008), but, few studies have empirically investigated variation in adrenarche across populations. While other early life stages, such as the prenatal growth period and menarche, are marked by culturally compelling and easily discernible factors (birth size and first menses, respectively), adrenarche is viewed more as a silent physiological phenomenon (Havelock et al. 2004). I argue that adrenarche, a measurable hormonal event, merits recognition as a marker of life history strategy. For the purpose of this thesis, adrenarche refers to the maturation of the adrenal gland, and is marked by a steep rise in adrenal androgen production, namely DHEAS levels. The point at which these levels rise above 40 µg/dl is the technical definition of children having reached adrenarche (Wierman et al. 1986).

Synthesizing research regarding juvenility from different disciplines suggests that other changes also occur around the time of adrenarche. Adrenarche occurs in humans after the completion of brain growth in weight and the eruption of the first permanent molar (Bogin 2001), but around the same time as the adiposity rebound (Remer 2000). These morphological changes occur simultaneously with changes in behaviour. Around the same time adrenarche occurs, increases in a child's cognitive ability, food processing and participation in chores lead to increased social interaction (Weisner 1987; Bogin 2001; Campbell 2006). In turn, adults begin to "notice" juveniles as capable of taking on social responsibilities and tasks (Lancy and Grove 2011). These morphological and behavioural changes may help to explain why adrenarche evolved, however their causal links with adrenarche still need to be strengthened.

Life history theorists have proposed that humans have evolved to be sensitive to specific features of early childhood environments and that exposure to different environments biases children toward development of different reproductive strategies (Charnov and Berrigan 1993, Ellison 1996, Bogin 2001). For example, in ecologically-favourable environments, girls reach the age of sexual maturity earlier, reflecting a faster reproductive strategy. Adrenarche may have evolved to promote cognitive development, store energy for later adolescent growth and accommodate social learning. An earlier age at adrenarche may also reflect a faster reproductive strategy.

Regardless of why it evolved, it is unknown whether the timing of adrenarche is relatively fixed or plastic. In a stable environment, evolutionary pressures operate to select traits that match the organism to its environment. If a change in environment occurs, traits that developed in response to the prior environment may be mismatched with current ecological conditions. If the change in environment occurs during a period of plasticity, the timing of development may be altered to match current ecological conditions. It has been speculated that adrenarche entails plasticity in adapting to energy resources, other environmental cues and the social needs of adolescence and their maturation (Hochberg 2010). However, very little empirical evidence demonstrating actual variation in adrenarche exists.

The expectation of adrenarche occurring around age seven is based largely on hormone profiles developed from clinical studies among industrialised populations (Korth-Schutz, Levine et al. 1976; Reiter et al. 1977). Industrialised populations have access to health care, calorically dense market foods, and have infant mortality levels of less than 1% (Kramer and Greaves 2011). Such resources represent an ecologically abundant environment which is evolutionarily recent and novel. On the contrary, children living in ecologically stressed societies grow up in environments with fluctuations in food supply, under harsh infectious disease conditions, with little or no access to market foods, health care, or immunization

(ibid). Given these differences and the known impact that early life conditions have on child development at large, the timing of adrenarche also should be expected to vary. For children growing up in conditions reflecting the nutritional and epidemiological transition, the timing of adrenarche may be rapid or protracted compared to pre- and post-industrialised populations, respectively. Although explicit attention has been given to cross-cultural differences in adolescent growth and puberty, far less is known about developmental variation during juvenility.

Within clinical populations, studies have demonstrated an earlier age at adrenarche and elevated adrenal androgens among small-for-gestational-age babies (Ibáñez et al. 2000). Clinical studies also suggest that premature adrenarche may be a forerunner of Metabolic Syndrome, which includes polycystic ovary syndrome (PCOS) among other morbidities (obesity, hypertension, insulin resistance, type 2 diabetes, and dyslipidaemia) (Ibanez et al. 2000). An expanding body of evidence points to the relevance of adrenarche in influencing adult reproductive function even among healthy individuals (Apter et al. 1989; Núñez-de la Mora et al. 2007). Núñez-de la Mora and colleagues demonstrated (2007) that the environment experienced during the juvenile period affects adult reproductive hormone profiles. In a migrant study of healthy women of reproductive age (19 – 35 years), women who migrated from Bangladesh to England as children had higher luteal progesterone levels than women who migrated as adults. Bangladeshi women specifically, who migrated to the UK before age eight years, display the highest progesterone concentrations compared to women who migrated after age eight years. Their results point to the period before adrenarche as a time sensitive to environmental factors that influence the production of adult sex steroids later in life. Whether this critical window is important in the timing of adrenarche has not been previously investigated.

ADRENARCHE AS A CRITICAL WINDOW FOR REPRODUCTIVE FUNCTION

Juvenility is hypothesised to be a time of hyper plasticity endowed with predictive adaptive responses of body and brain to the environment (Hochberg 2008). Similarly, adrenarche is proposed to be a critical window in affecting the trajectory of reproductive function as demonstrated in the hormonal profiles of Bangladeshi women who migrated as children (Núñez-de la Mora et al. 2007). The latter study prompted me to ask whether the timing of adrenarche and levels of androgens also differ among Bangladeshi, British-Bangladeshi and British girls. If androgen levels and the timing of adrenarche differ among migration groups, then environmental factors before middle childhood may affect developmental timing. If adrenarche does not differ between these migration groups, then the periods either before adrenarche, or after adrenarche and before menarche, may be periods of plasticity. A migrant study provides a novel situation to test whether the timing of adrenarche varies by ethnicity, ecology or migration.

The ABBY Project was designed to recruit girls aged of 5 – 16 years from primary and secondary schools in Sylhet, Bangladesh and London, UK. Comparing Bangladeshi and British-Bangladeshi girls in a migrant model is an ideal situation in which to assess whether the developmental environment is associated with the timing of adrenarche because 95% of British-Bangladeshi migrants originate from Sylhet, the northeast region of Bangladesh (Gardner 1995). They are almost uniformly Muslim and intermarriage outside of their community is rare (ibid). Also the developmental environment in Bangladesh differs greatly from that in the UK specifically with regard to sanitation and access to health services which are all likely to increase infectious disease burden. However, Nunez de la More et al. (2007) have previously reported that the migrants come from relatively high socioeconomic classes

in Sylhet, meaning they are comparable to British-Bangladeshis in the UK with regard to nutritional status. At the same time, identity groups within the British and Bangladeshi populations may hold different values concerning their food environments (Ulijaszek 2007).

HYPOTHESIS AND PREDICTIONS

I compare the timing of adrenarche according to ethnicity, ecology and migration previously outlined in Chapter 1. If juvenility differed by ethnicity all Bangladeshi girls would reach adrenarche at a different age than white British girls. If juvenility differed by migration, girls would increasingly reach adrenarche earlier with increasing individual/ancestral generations lived in the UK. If juvenility differed by country-level ecology Sylheti girls would reach adrenarche later than all girls living in the UK. If juvenility differed by discontinuity in ecology, first generation girls would reach adrenarche earlier than all girls. I tested the following hypothesis and predictions among Bangladeshi and British girls which subscribes to the ecological model of development.

Hypothesis: Adrenarche as measured by DHEAS, juvenile secondary sex characteristics and growth will differ according to ecology rather than ethnicity or migration scale. First generation British-Bangladeshi girls, who spend their early life in Bangladesh and then move to the UK, will reach adrenarche at an earlier age, report more somatic signs of adrenarche, will grow faster and show higher DHEAS than Bangladeshi girls who live in Sylhet and second generation and white British girls who live in the UK.

- Prediction A: DHEAS concentrations will be lower in Sylheti girls than in second generation British-Bangladeshi and white British girls and DHEAS production will be higher among first generation British-Bangladeshi girls compared to all other girls.
- Prediction B: The median age at adrenarche will be older in Sylheti girls than in second generation British-Bangladeshi and white British girls and younger in first generation British-Bangladeshi compared to all other girls.
- Prediction C: Anthropometric measures including heights, weights, BMI and waist circumference will increase across the migration groups in the following order: Sylheti, second generation British-Bangladeshis and white British girls, first generation British-Bangladeshis.
- Prediction D: The prevalence of juvenile secondary sex characteristics (underarm hair, lower leg hair, pimples, oily skin and body odour) will increase in the following order: Sylheti, second generation British-Bangladeshis, white British girls and first generation British-Bangladeshis.

METHODS

PARTICIPANTS

Out of a total of 488 girls who were enrolled into the study, 470 girls provided saliva specimens, and from these 418 (Sylheti= 164, first generation = 42, second generation = 162, white British= 50) provided complete pubertal stage and anthropometric data. Recruitment techniques and ethics permission have been previously described in Chapter 2.

MEASUREMENTS

QUESTIONNAIRE

The study questionnaire assessed variables regarding family-level socio-demographic measures as described in Chapter 2. Pubertal staging was assessed via self-report using the PDS-A which included five questions regarding juvenile secondary sex characteristics, the details of which are described in Chapter 2 (Appendix 4).

SALIVA SPECIMENS

Details of saliva collection are included in Chapter 2. In this Chapter, a total of 470 saliva samples in duplicate were analysed for DHEAS and included in statistical analyses. Overall coefficients of variation for duplicate samples were less than 20% and the manufacturer's lower and upper limits of quantitation were 43 pg/ml and 16,000 pg/ml respectively (Salimetrics ©).

Anthropometrics

All measurements were taken according to standardised methods as outlined in methods Chapter 2. Anthropometric measurements, including height, weight, and waist circumference, were taken while the participant was clothed but without shoes and bulky outer clothing.

Body mass index (BMI) was calculated by dividing weight in kilograms by stature in metres squared. Because BMI does not distinguish between fat and fat-free mass, waist circumference measurements were used to assess abdominal fat (Lohman et al 1988).

Statistical Analyses

DHEAS concentrations were log transformed to reduce positive skewness. None of the independent variables were transformed.

For the analyses testing for variation according to migration scale, a dummy variable representing individual/ancestral generations living in the UK was created. Migration scale was coded 0 to 3 with 0 for Sylhetis, 1 for first generation British-Bangladeshi, 2 for second generation British-Bangladeshi, and 3 for white British.

Growth

Standard multiple linear regressions were used to evaluate differences in anthropometric measurements by migration group. Height, weight, BMI and waist circumference were compared among all girls aged less than 9.5 years. This was also the median age of all girls in the sample and the age when 90% of girls had reached adrenarche.

Height-for-age and weight-for-age z-scores were calculated and categorised into quartiles. Age-specific quartiles for waist circumference were determined by placing each girl into age-appropriate categories. BMI z-scores were also calculated for each girl and compared to UK growth references to determine the percentage of girls who were clinically underweight,

normal weight, overweight, or obese (Cole and Green 1992; Cole et al. 1998). It is standard to determine wasting or obesity by marking girls +/- two standard deviations from the mean (upper and lower 2.5%) and, by classifying girls this way, most girls were classified as normal weight.

DHEAS

DHEAS was compared among groups and age-quartiles using ANOVA. Standard multiple linear regressions were used to evaluate differences in DHEAS among migration groups and to analyse group differences in hormone levels adjusting for age and anthropometrics. DHEAS was the dependent variable, while migration scale, age, height, weight, BMI and waist circumference were independent variables.

Adrenarche Status

Adrenarche is clinically defined when DHEAS levels exceed 40-50µg/dl in serum (Wierman et al. 1986). The threshold was converted to reflect the concentration found in saliva. Saliva contains 0.1% of the DHEAS found in plasma. Thus, 40 µg/dl of DHEAS in serum equals 400 pg/ml of DHEAS in saliva. As a result, a binary variable was created coding all values below and above 400 pg/ml as 0 and 1, respectively. This variable is referred to as adrenarche status; 0 reflects that the girl has not reached adrenarche and 1 reflects that she has reached adrenarche. Adrenarche status was used to model age at adrenarche.

Timing of Adrenarche

Median age at adrenarche (as defined by adrenarche status) was estimated using Weibull regression models for parametric survival analysis. This model accounts for the double censoring present in the cross-sectional data collected here: some of the girls had not yet reached adrenarche at her current age (right-censored) or had reached adrenarche at some unknown age in the past (left-censored). Goodness-of-fit of the Weibull regression models

was assessed by graphical inspection of the resulting survival curve for age at adrenarche for each migration group against the survival curve based on a non-parametric survival estimate akin to the Kaplan-Meier estimator (Appendix 6). The median ages at adrenarche for each migration group and anthropometric quartiles were then derived from the Weibull regression models. Using the Weibull regression models, I created plots for the distribution density of age at onset of adrenarche for each migration group.

I modelled age at adrenarche twice: the first model included all girls; the second model only included those girls younger than age 9.5 years. I focus on those results for models with girls younger than age 9.5 years because more than 90% of girls had reached adrenarche by that age. Older girls were at least three years post adrenarche, so their DHEAS levels and anthropometrics are less informative about the relationship between DHEAS and body size regarding the onset of adrenarche.

Markers of Adrenarche: Juvenile Secondary Sex Characteristics

Responses to the PDS-A were transformed into binary variables to indicate the presence or absence of juvenile secondary sex characteristics. Using both logistic and the Weibull regressions as described above, the median age at adrenarche using each marker was compared among migration groups. For all girls the median age at adrenarche, as defined by DHEAS, was compared to the median age of each juvenile secondary sex characteristic. All statistical analyses were conducted using Stata Version 11.

RESULTS

Data from 418 girls were analysed to investigate adrenarche and juvenile characteristics. The number of girls varied by migration group and age: 164 Sylheti girls, 42 first generation British-Bangladeshi, 162 second generation British-Bangladeshi and 50 white British. Table 4 outlines the age distribution for each migration group among those girls with DHEAS values.

Table 4: Distribution of Age in Each Migration Group Among Girls with DHEAS Values

Age (years)	Bangladesh	First Generation	Second Generation	British	Total
4	1	0	0	0	1
5	10	0	5	3	18
6	15	7	14	3	39
7	33	5	27	4	69
8	24	3	23	10	60
9	13	4	11	5	33
10	14	3	20	7	44
11	16	2	20	9	47
12	6	6	15	4	31
13	16	3	10	2	31
14	6	3	10	1	20
15	6	4	6	2	18
16	4	2	1	0	7
Total	164	42	162	50	418

GROWTH AT ADRENARCHE

Migration group differences in anthropometrics are described in Table 5. Height, weight, waist circumference, and BMI increased with individual/ancestral generations lived in the UK. Additional multiple linear regressions testing for differences in anthropometrics according to migration group demonstrated that among girls at the same age, all groups of girls living in the UK were larger in body size when compared to Sylhetis.

Table 5: Summary of sample and anthropometrics comparing Sylheti, First Generation, Second Generation and White British Girls Less Than Age 9.5 Years

Migration Group									
Variables	Sylheti		First Generation		Second Generation		White British		ANOVA p-Value
N	93		19		74		23		
Age (years)	7.4†	(1.2)	7.5	(1.1)	7.6	(1.0)	7.6	(1.3)	0.5
Height (cm)	116.4†	(9.8)	123.6	(8.9)	124.8	(9.9)	126.5	(7.9)	<0.0001
Weight (kg)	20.5†	(5.8)	28.4	(10.0)	28.0	(8.3)	29.6	(6.4)	<0.0001
Body Mass Index (kg/m²)	15.0†	(3.0)	18.2	(4.2)	17.6	(3.1)	18.3	(2.5)	<0.0001
Waist Circumference (cm)	48.9†	(6.4)	59.8	(9.4)	58.7	(7.4)	61.0	(5.4)	<0.0001
BMI Z-Score	-0.87†	(1.37)	0.66	(1.70)	0.53	(1.28)	0.95	(1.10)	<0.0001
Nutritional Status*									
Underweight	47%		16%		8%		4%		
Normal Weight	46%		47%		62%		39%		
Overweight	4%		16%		21%		48%		
Obese	3%		21%		10%		9%		

Values given as mean (SD).

*Nutritional Status is derived from BMI z-scores and related to UK Clinical references

†Pair wise comparisons showed that Sylhetis were significantly different from each groups living in the UK, but no other groups were significantly different from each other.

DHEAS BY AGE AND MIGRATION GROUPS

Salivary DHEAS levels increased with age across all migration groups. Table 6 summarises DHEAS for each migration group and age quartile. After adjusting for age, DHEAS levels did not differ across migration groups (p-trend=0.41).

Table 6: Comparison of log DHEAS (mean and standard deviation; pg/ml) Levels Among Migration Groups using ANOVA.

	Migration Group								
Variables	Sylheti		First Generation		Second Generation		White British		ANOVA P-value
Age Quartile									
1 (<7.5 yr.)	5.3	(1.4)	5.98	(.8)	5.78	(1.2)	5.41	(.7)	0.23
2 (7.5 – <9.5 yr.)	6.22	(1.2)	6.95	(1.1)	6.36	(1.1)	6.39	(1.0)	0.42
3 (9.5 - <11.8 yr.)	7.28	(.7)	6.89	(.9)	7.14	(1.0)	7.25	(1.0)	0.79
4 (>11.8 yr.)	7.82	(.8)	7.57	(.8)	7.76	(.9)	7.67	(.9)	0.75

A lowess smoothing curve of DHEAS by age showed an inflection in levels of DHEAS along the ages 5 and 7 years and 5.5- 6 log DHEAS pg/ml (Figure 24). The point of inflection corresponded with the clinical cut point of 400pg/ ml of DHEAS (5.9 on the log scale) that indicates adrenarche. Figure 25 fits a line of the natural log of DHEAS by age for each migration group and illustrates that DHEAS levels were slightly higher among those girls living in the UK compared with those living in Sylhet, and, with the exception of first generation migrants, the differences levelled out around age 12.

Figure 24: Lowess Smoother of the Log of DHEAS by Age among all girls aged 5-16 years.

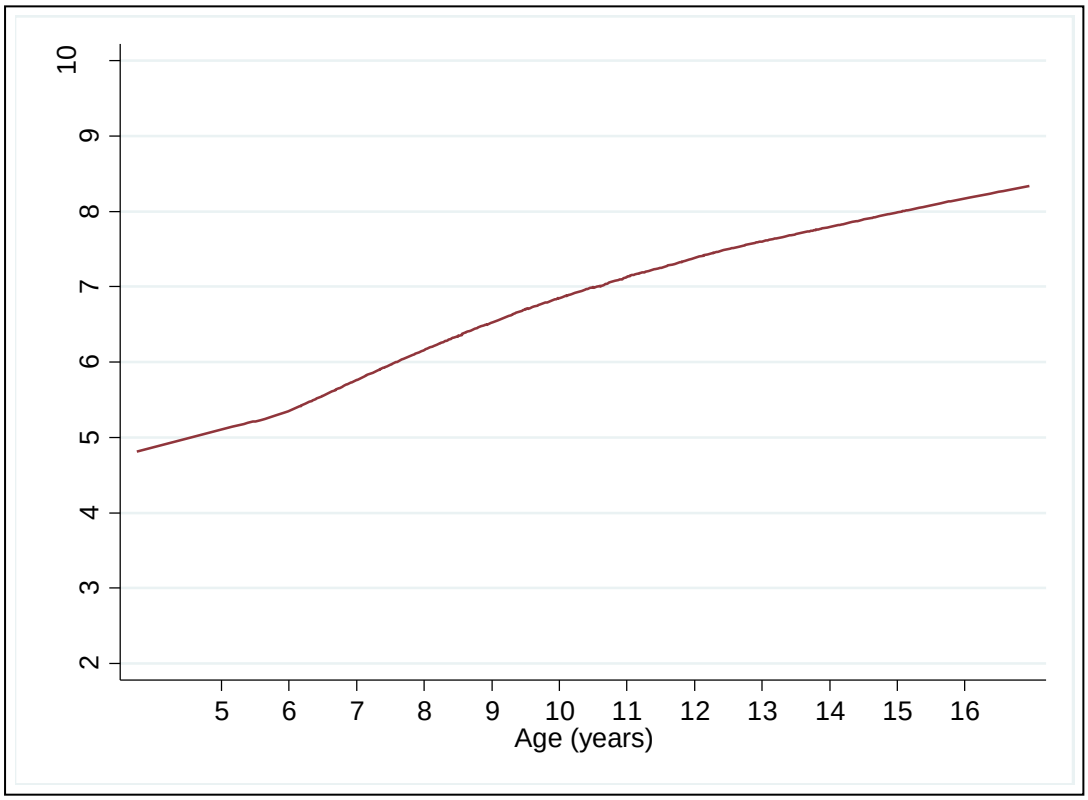
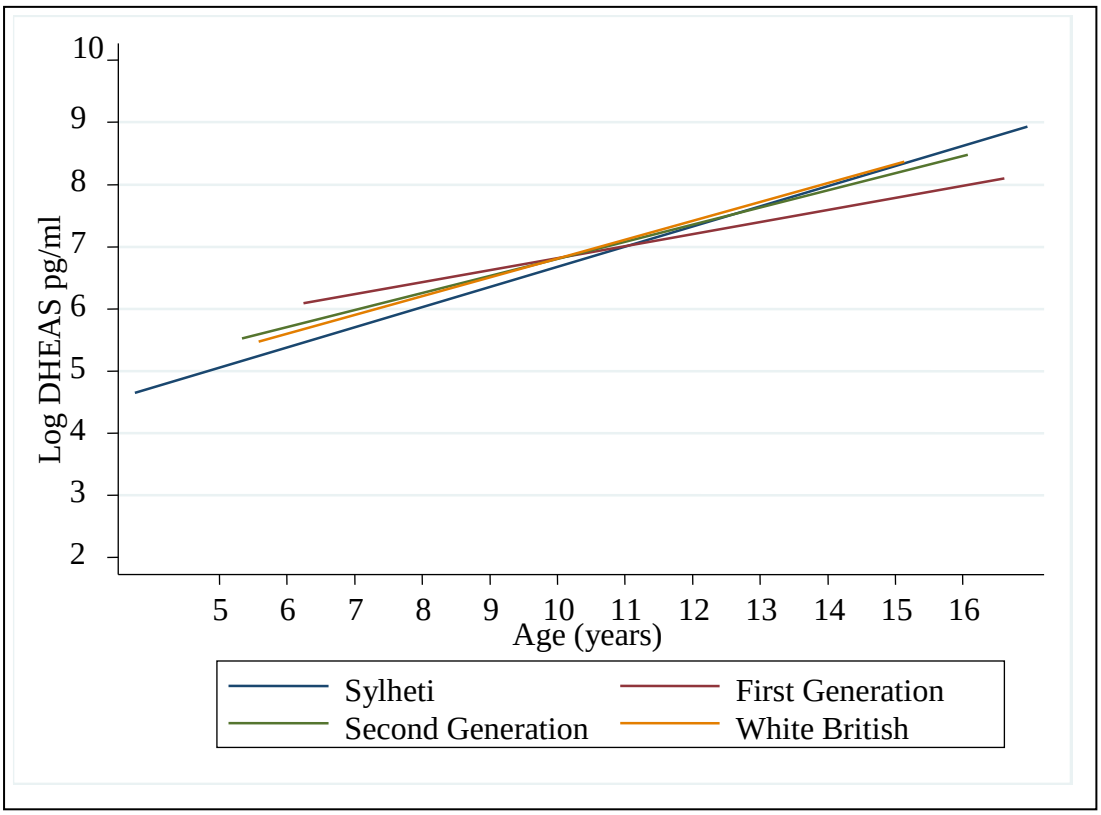


Figure 25: Linear Fit of the Log of DHEAS by Age for Migration Groups

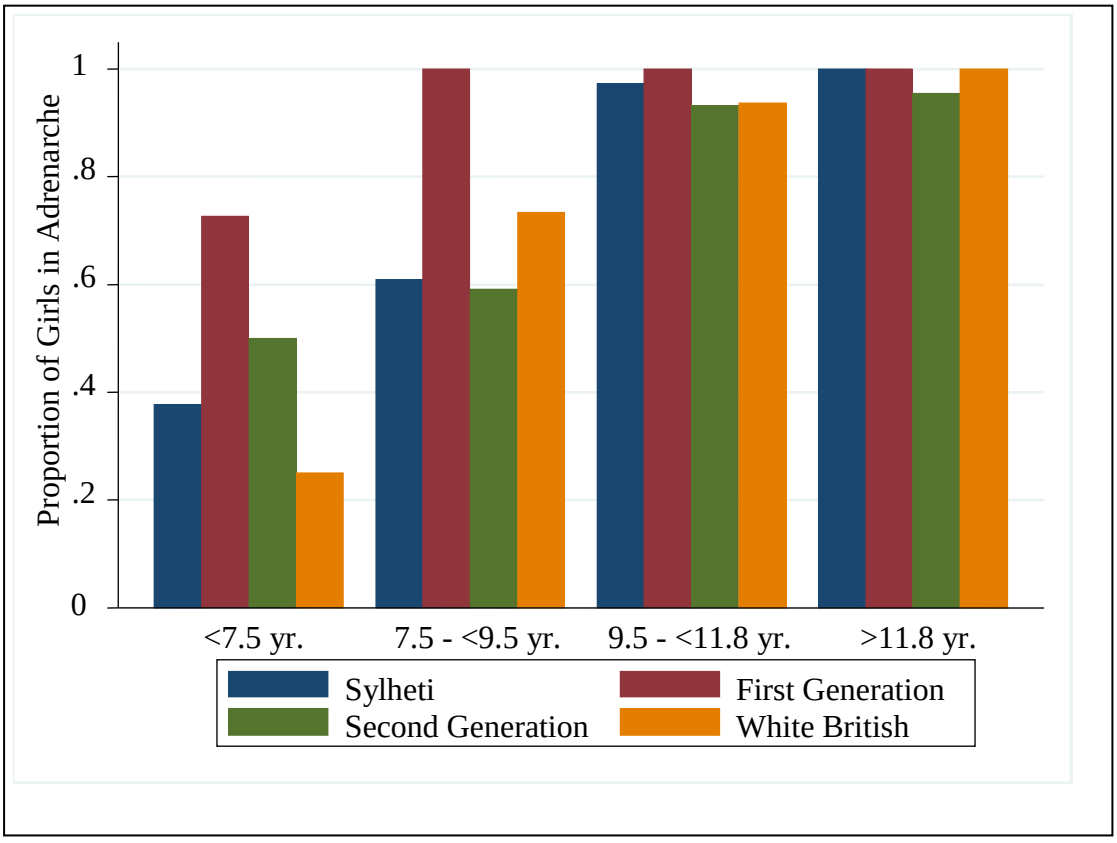


DHEAS and Adrenarche Status

When adrenarche status was determined by salivary DHEAS levels above 400 pg/ml, 76% of the total sample had reached adrenarche. There was variability in the timing of adrenarche as some girls at age five years had reached adrenarche, while others at age 12 years had not.

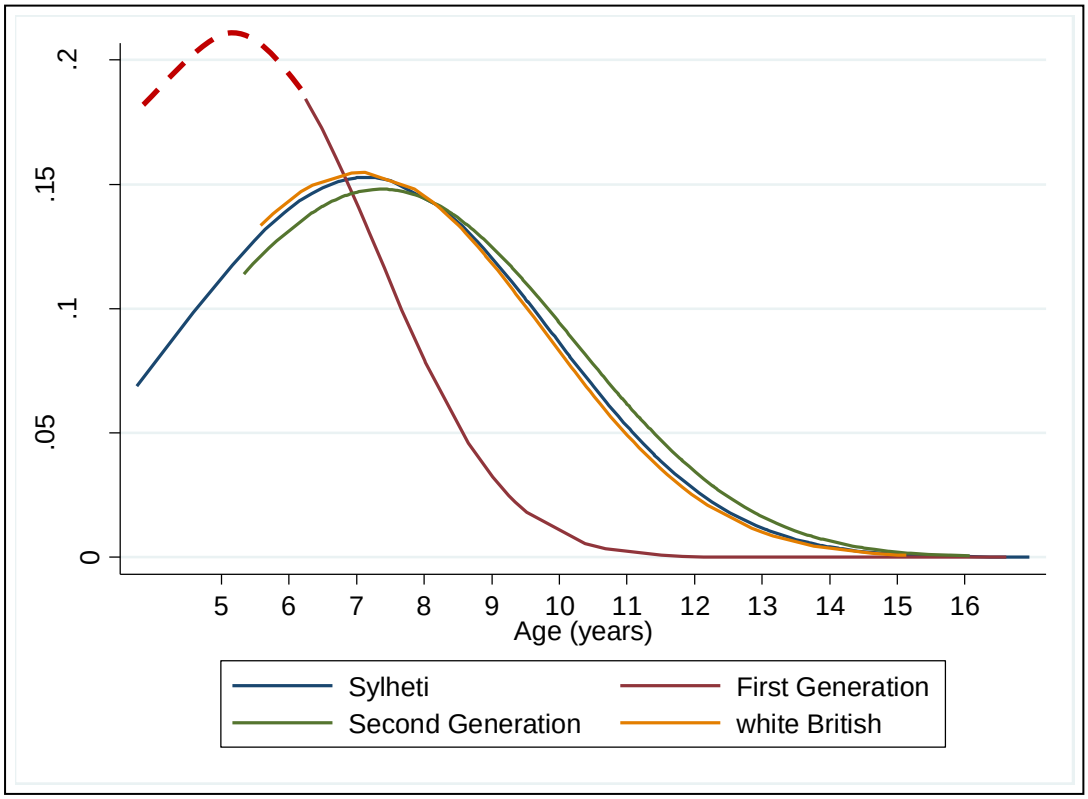
Figure 26 illustrates the proportion of girls for each age quartile who had reached adrenarche. In each migration group, 100% of girls had reached adrenarche by the following ages: Sylhetis = 10 years, first generation = 8 years, second generation = 13 years, and white British = 9 years.

Figure 18: Comparison of the Proportion of Girls in Adrenarche (>400pg/ml DHEAS) by Age Quartile and Migration Group.



The median ages at adrenarche by migration group were Sylheti = 7.2, first generation = 5.3, second generation = 7.4, WB = 7.1, and they did not decline by migration scale (p-trend= 0.7171). The median age at adrenarche for first generation British-Bangladeshi girls was about two years before all other migration groups (p= 0.003) (Figure 27). The differences in median age at adrenarche between each migration group were statistically significant at the p<0.001 level of significance.

Figure 27: Comparison of the Distribution Density of the Age at Onset of Adrenarche among Sylheti, First Generation, Second Generation and White British Girls Determined by Weibull Regression Models for Parametric Survival Analysis.



The median age at adrenarche for each migration group was: S= 7.2, BB1= 5.3, BB2= 7.4, WB = 7.1; p-trend=0.7). As seen by the red line, first generation girls reached adrenarche much earlier than all other girls. The dotted red line projects the distribution density for girls younger than age six years.

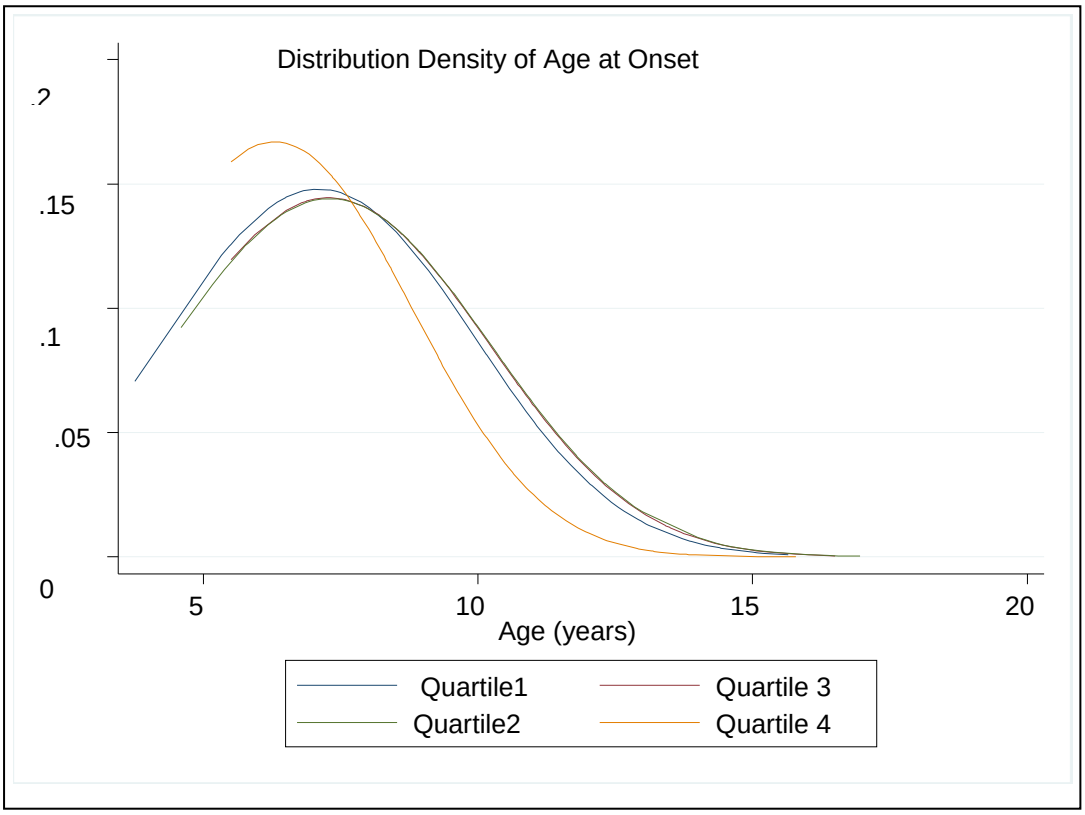
The timing of adrenarche attainment was significantly associated with height (p-trend= 0.002) and weight (p-trend= 0.005), but waist circumference did not significantly change the timing of adrenarche (p=0.142) (Table 7). The association of BMI z-score quartiles with

adrenarcheal timing was borderline significant (p=0.06) (Table 7). The median age at adrenarche was about one year earlier among girls with a BMI z-score in the upper-most quartile (Figure 28). The possibility of BMI and migration group interacting was explored by modelling an interaction term; however, the study did not have sufficient power to draw any conclusions. Therefore, median age at adrenarche by BMI quartile was stratified by migration group and the results are presented in Table 8. The association of BMI with adrenarche was most apparent in all Bangladeshi girls (Table 8), although there was no statistical evidence that the association of BMI with age at adrenarche varied by migration group (p= 0.32).

Table 7: Comparison of Age at Adrenarche Among Quartiles of Height, Weight, BMI and Waist Circumference using Weibull Regression Models for Parametric Survival Analysis

Adrenarche	HR	95% CI		p-value	Trend
Height for Age Z-score					<0.01
<25%	1				
25-50%	1.0	(0.5 -	1.9)	0.88	
51-75%	1.5	(0.8 -	2.7)	0.13	
>75%	2.9	(1.6 -	5.1)	<0.01	
Weight for Age Z-score					<0.01
<25%	1				
25-50%	0.9	(0.6 -	1.7)	0.85	
51-75%	2.3	(1.3 -	2.5)	<0.01	
>75%	1.8	(1.0 -	3.0)	0.03	
BMI Z-Scores					0.06
Quartile 1	1				
Quartile 2	1.0	(0.8 -	1.3)	0.99	
Quartile 3	1.1	(0.9 -	1.4)	0.24	
Quartile 4	1.1	(1.0 -	1.3)	0.10	
Waist Circumference Age-Specific Quartile					0.09
<25%	1				
25-50%	0.9	(0.5 -	1.6)	0.75	
51-75%	1.4	(0.8 -	2.3)	0.24	
>75%	1.6	(1.0 -	2.6)	0.07	

Figure 28: Comparison of the Distribution Density of Age at Onset of Adrenarche by BMI Z-score Quartile determined by Weibull Regression Models for Parametric Survival Analysis



The median age at adrenarche for each BMI z-score quartile was: Quartile 1= 7.1, Quartile 2 = 7.3, Quartile 3 = 7.3, Quartile 4 = 6.5; p-trend = 0.2. As seen by the yellow line, the girls with BMI above the 75th percentile reached adrenarche much earlier than all other girls.

Table 8: Median Age at Adrenarche Stratified by BMI Z-score Quartile and Migration group Among Girls Younger than Age Ten Years

BMI Z-Score	Sylheti	First Gen.	Second Gen.	White British
Quartile 1	7.4	7.5	9.2	7.7
Quartile 2	6.7	6.7	7.5	7.6
Quartile 3	6.0	6.0	6.1	7.5
Quartile 4	5.4	5.4	5.0	7.3

MARKERS OF ADRENARCHE

JUVENILE SECONDARY SEXUAL CHARACTERISTICS AND MIGRATION GROUP

There were some statistically significant differences in pair-wise comparisons in the prevalence of lower leg hair and pimples, with girls who were born in the UK reporting these characteristics at earlier ages compared with Sylheti girls [OR (95% Confidence Interval) for Lower Leg Hair: Sylheti = 1, 2nd Gen = 1.8 (1.1- 2.9), white British = 2.3 (1.1- 4.7); and Pimples: Sylheti = 1, 2nd Gen = 3.3 (2.0- 5.7), white British = 5.5 (2.6- 11.7)] (Table 9). Body odour occurred earlier in Sylheti girls when compared with first and second generation British-Bangladeshi girls. [OR (95% Confidence Interval): Sylheti = 1, 1st Gen = 0.2 (0.1-0.5), 2nd Gen = 0.4 (0.2-0.6)]. The prevalence of juvenile characteristics (underarm hair, lower leg hair, pimples, oily skin and body odour), increased with increasing individual/ancestral generations lived in the UK, even after adjusting for age, height and weight. Odds ratios for lower leg hair and pimples increased with migration scale, after adjusting for age: leg hair (ORs = 1.0, 0.4, 1.8, 2.3; p-trend = 0.003), pimples (ORs = 1.0, 1.7, 3.3, 5.5; p-trend < 0.0001). For body odour, odds ratios decreased with increasing individual/ancestral generations lived in the UK (ORs = 1, 0.21, 0.35, 0.59; p-trend < 0.0001). There was no significant trend for oily skin (ORs = 1, 0.6, 1.1, 1.3; p= 0.89) or underarm hair (ORs = 1, 0.56, 0.75, 0.72; p= 0.20) (Table 9).

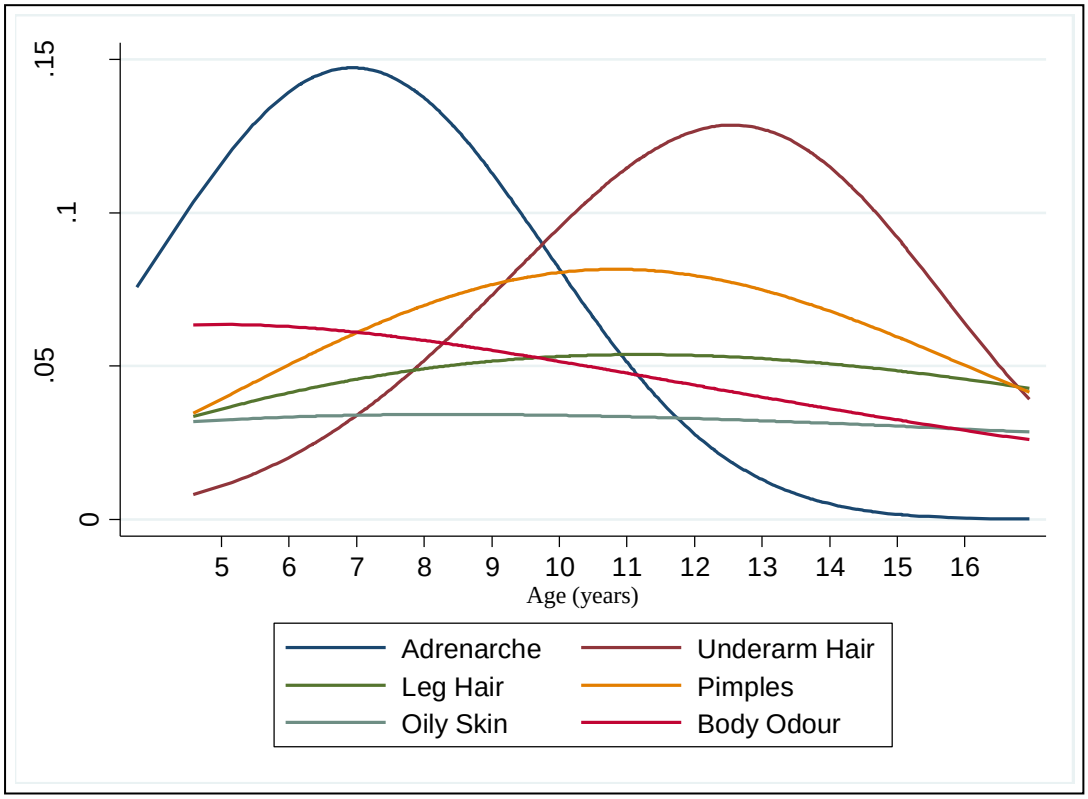
Table 9: Comparisons of the Age of Development for Juvenile Characteristics Among Sylheti, First Generation, Second Generation and White British Girls Using Multiple Logistic Regressions adjusted for age.

Characteristic	Group	Odds Ratio	95% Confidence Intervals		p<0.05
Underarm Hair					
	Trend	**0.88**	**0.73**	**1.07**	
	Sylheti	1.00			
	1st Generation	0.56	0.22	1.39	
	2nd Generation	0.75	0.43	1.33	
	white British	0.72	0.31	1.63	
Lower Leg Hair					
	Trend	**1.27**	**1.07**	**1.51**	*****
	Sylheti	1.00			
	1st Generation	0.39	0.15	1.00	
	2nd Generation	1.75	1.10	2.90	*
	white British	2.30	1.10	4.70	*
Pimples					
	Trend	**1.59**	**1.32**	**1.90**	*****
	Sylheti	1.00			
	1st Generation	1.65	0.70	3.89	
	2nd Generation	3.34	1.97	5.67	*
	white British	5.49	2.58	11.67	*
Oily Skin					
	Trend	**0.99**	**0.83**	**1.17**	
	Sylheti	1.00			
	1st Generation	0.64	0.27	1.53	
	2nd Generation	1.08	0.66	1.77	
	white British	1.32	0.65	2.69	
Body Odour					
	Trend	**0.80**	**0.68**	**0.93**	*****
	Sylheti	1.00			
	1st Generation	0.21	0.09	0.46	*
	2nd Generation	0.35	0.22	0.57	*
	white British	0.59	0.30	1.17	

TIMING OF ADRENARCHE AND JUVENILE CHARACTERISTICS

The median age at adrenarche was compared to the median ages of each juvenile secondary sex characteristic (Figure 29). Adrenarche appeared before all juvenile characteristics, which followed in this order: body odour, pimples, underarm hair, lower leg hair, oily skin.

Figure 29: Comparison of the Distribution Density of Adrenarche and Juvenile Characteristics determined by Weibull Regression Models for Parametric Survival Analysis



The medians for age at onset for each juvenile characteristic were: adrenarche = 7.0, body odour = 9.0, pimples = 11.2, under arm hair = 12.2, leg hair = 13.2, oily skin= 16.4.

DISCUSSION

It is unknown whether the timing of adrenarche varies much across individuals or populations and whether levels of adrenal androgens produced during middle childhood similarly differ. The existing and limited literature suggests that the timing of adrenarche is relatively fixed among individuals except in pathological states where a child is said to have premature adrenarche. The study here suggests that there is more variation in the timing of adrenarche among healthy individuals than previously thought: energetic status as measured by BMI may be an important mediator of this timing.

PRODUCTION OF DHEAS AT ADRENARCHE

The results from ABBY do not support the hypothesis that continuous DHEAS levels differ significantly by ethnicity, ecology or migration. However, DHEAS increased with age among all Bangladeshi and British girls, as previously demonstrated among children of European descent (Reiter et al. 1977). In the ABBY study, salivary DHEAS was detectable in some girls before age five years, which agrees with a previous study that found overall androgen production (measured by the sum of urinary androgens and androgen metabolites) as early as age three years (Remer et al. 2005). Such early detections of salivary DHEAS support the view that adrenal androgen production is a developmental process that varies across individuals. When DHEAS levels from all girls were plotted by age, an increase in rate of DHEAS production was observed and marked by a point of inflection (Figure 24). This point of inflection corresponded with the clinical threshold established among other populations (Reiter et al. 1977, Wierman et al. 1986). While Remer and colleagues (2005) demonstrated a more gradual increase in the sum of total androgens and metabolites, they also found a marked rise in DHEAS. ABBY results show that the rate of adrenal androgen production increased around age seven years among all girls suggesting that, at the population level, adrenarche could be detected at a certain threshold when there is increased adrenal androgen production. This finding is limited as it is not based on individual longitudinal data.

Statistically, mean levels of DHEAS did not differ according to migration scale, yet descriptive data suggests that between migration group comparisons of DHEAS may differ depending on age. Among girls younger than age 9.5 years, DHEAS was highest among the first generation British-Bangladeshi girls and levels decrease in the following order: second generation, white British and then Sylheti girls. After age 9.5 years, first generation girls had the lowest levels of DHEAS and Sylhetis had the highest, with second generation and white British girls in-between. It is possible that the rate of adrenal androgen production changes

with age or that the activation of the HPO axis (which occurred around age nine years in most groups) affects DHEAS levels. A previous study, measuring androgens among American girls of different ethnicities, also noticed that while African- American girls had higher levels of androgens before age ten, there were no differences in androgens after age ten (Pratt et al. 1990). It has been suggested that once puberty begins, the adrenal androgens are tempered by other hormonal changes (Martin et al. 2004). The next Chapter will explore the relationship between adrenarche and puberty.

Plasticity of Adrenarche

The ABBY data support the hypothesis that age at adrenarche is advanced with discontinuity in developmental ecology. Earlier adrenarche was associated with discontinuity in country-level ecological conditions between Bangladesh and the UK. While adrenarche occurred around age 7 years for Sylheti, second generation and British girls, first generation migrants reached adrenarche two years before all the other populations. The similarity in age at adrenarche among Sylheti and British girls suggests that the timing of adrenarche is relatively consistent between ethnicities (at least those under study). But the timing of adrenarche can be altered when there is discontinuity in ecological factors during early childhood, as evident by the first generation migrant girls who moved to the UK on average at age 5.5 years. The timing of adrenarche returned to age seven among the second generation girls. Collectively, these findings suggest that the timing of adrenarche may be a relatively fixed process, but that a change in country-level ecological factors elicits a plastic response.

GROWTH DURING ADRENARCHE

Girls living in the UK were taller, heavier, thicker and more overweight/obese than Sylheti girls. According to all anthropometrics, first generation British-Bangladeshi girls were more similar to second generation and white British girls than Sylheti girls, suggesting that discontinuity in ecology during the juvenile period could affect growth relatively abruptly.

Age at adrenarche is influenced by energetic status as measured by BMI. The overall relationship between BMI and androgen levels is positively associated. Adrenarche occurred one year earlier among all girls above the 75^{th} percentile of BMI z-scores. Remer et al. (2000) have previously demonstrated that an increase in BMI during the adiposity rebound is associated with the onset of adrenarche.

The association of BMI with the timing of adrenarche may differ by ethnicity. Although age at adrenarche decreased with increasing BMI and BMI increased with migration scale, there was no evidence that the age at adrenarche decreased by increasing migration scale. When the survival model that tested for differences in adrenarcheal timing among groups was adjusted for BMI, first generation girls still reached adrenarche earlier than the other groups suggesting that energetic status does not explain all the variation in the timing of adrenarche. Descriptively, the interaction of BMI and adrenarche was different between Bangladeshi and British girls. Among white British girls there were no marked changes in age at adrenarche with increasing BMI, but among all Bangladeshi girls (regardless of living in Bangladesh or the UK), age at adrenarche declined with increasing BMI. White British girls had higher BMI than all other girls yet they did not have the earliest age at adrenarche. BMI may be measuring different aspects of lifestyle among the migration groups. BMI of first generation girls, who collectively experienced early adrenarche, was markedly higher than Sylheti girls; therefore a rapid change in nutritional status may affect adrenarcheal timing.

MARKERS OF ADRENARCHE

When juvenile secondary sex characteristics were used to assess the timing of adrenarche across migration groups, the patterns differed compared to DHEAS-defined adrenarche. The prevalence of lower leg hair and pimples increased with migration scale, whereas, the prevalence of body odour decreased with migration scale. Using lower leg hair or pimples as a marker of adrenarche suggests that white British girls reached adrenarche earlier than all Bangladeshi girls. Using body odour as a marker pointed to Sylhetis as having an earlier adrenarche. Assessing adrenarche by DHEAS alone, the gold standard, suggests that the timing of adrenarche only differs among first generation migrants. Despite having the highest DHEAS levels, the age-adjusted prevalence of all juvenile characteristics was lowest among the first generation. It is possible that the migration groups differ in their skin sensitivity to androgen hormones or that the rate of androgen production affects the expression of secondary sex characteristics.

Adrenarche status, as defined by DHEAS, occurred before the appearance of all of the secondary sexual characteristics. Whether the trends in earlier juvenile secondary sex characteristics are the result of adrenal androgens cannot be determined by the cross-sectional study design. While the presence of juvenile sex characteristics were associated with higher levels of DHEAS levels (Chapter 2), differences in DHEAS levels did not account for the differences in secondary sex characteristics among migration groups.

The order of development of juvenile characteristics among all girls suggests that some juvenile secondary sex characteristics may be useful markers of juvenility in future studies where hormonal analyses are not feasible. Body odour appeared first and changes in sebaceous glands occurred before growth of sexual hair. Studies of the pilosebaceous units have demonstrated that sebaceous gland function begins before the initiation of pubic hair

growth at relatively lower levels of testosterone (Deplewski and Rosenfield 2000). Serum levels of DHEAS have been found to correlate with sebum production in early puberty (Stewart et al. 1992). In acne-prone areas of the skin, androgens cause the juvenile pilosebaceous unit to develop into a sebaceous follicle in which the hair remains vellus and the sebaceous gland enlarges tremendously. The opposite occurs in areas of the body that are sensitive to hair growth: androgens cause the pilosebaceous unit to develop into a hair follicle. The sensitivity of the hair follicle to androgens seems to follow a different dose-response curve than the sebaceous gland; the hair follicle is highly sensitive to dihydrotestosterone and the sebaceous gland is similarly sensitive to testosterone. The level of androgens needed for sexual hair growth may be higher than those necessary for sebaceous gland changes with lower levels being typical of middle childhood. Changes associated with the sebaceous glands, rather than sexual hair, may be a better marker of adrenarche for future studies.

In this study, underarm hair growth may be mediated by hormonal changes other than adrenal androgens. It has been proposed that adrenal androgens, independent of gonadal androgens, may drive axillary hair growth (Sizonenko and Paunier 1975). The most direct evidence that androgens are the principal hormones controlling sexual hair growth is that androgens given exogenously stimulate hair growth in eunuchs (Randall 1994). Axillary hair has been proposed to grow after pubic hair (Rosenfield 1986) which suggests that higher levels of androgens, perhaps as a result of ovarian production of androgens, may also drive axillary hair growth. Castration in men after puberty reduces but does not eliminate facial and axillary hair, suggesting that both adrenal and gonadal androgens are involved in hair growth (Hamilton 1960). Ovarian production of androgens may increase the amount of androgens available in the skin, but oestrogens may also play a role in sexual hair growth. Oestrogen in low dosage stimulates pubic and axillary hair growth slightly. For example, pubic hair

increases upon inducing puberty in hypogonadal patients with physiological doses of oestradiol alone (Rosenfield and Fang 1974). Thus, changes in sexual hair growth may be better markers of puberty, while changes in the sebaceous glands such as body odour and oily/pimply skin may be better markers of adrenarche.

LIMITATIONS OF THE STUDY

The timing of adrenarche may vary across populations, but the sample of girls studied here may not have been ideal to test the hypotheses. The small sample size among first generation and white British groups limit the interpretation and extrapolation of these results to the respective populations at large. Similarly, there was uneven distribution of age among the groups although the survival analyses were modelled to account for these differences.

The mismatch between earlier adrenarche according to energetic balance but not migration scale may be due to limitations of this study such as:

Few British-Bangladeshi or white British girls were clinically underweight. Even though white British girls had higher measures of adiposity than Bangladeshis, it may be that the difference in BMI was not large enough to pick up the association of energetic status with adrenarcheal timing. Replication of this study in other populations that differ vastly by energetic status may show more inter-population variation in adrenarcheal timing.

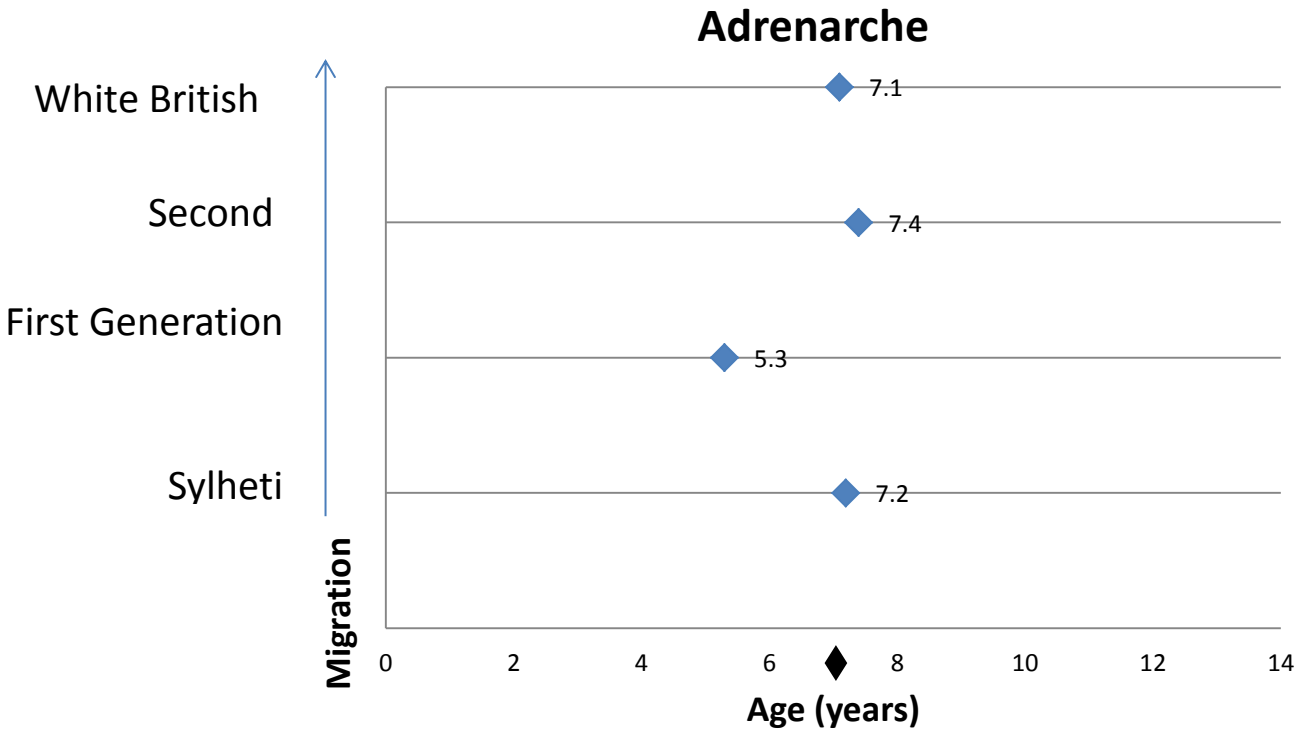
It is also possible that the first generation girls reached adrenarche earlier, not because of a change in environment accompanying migration, but because of a self-selection bias. This means that as a group they differ from Sylhetis and/or past migrants by socioeconomic, nutritional, immunological or psychosocial factors. I explore how first generation migrants differ from other Bangladeshis in Chapter 5.

The mismatch of DHEAS and juvenile characteristics as markers of adrenarche may be due to methodological constraints of the study. The cross-sectional design of the study meant that the age at which the juvenile characteristic appeared was unknown—what was known was that it had or had not appeared in the past. On the other hand, DHEAS levels corresponded with the exact age at the time of collection. The lack of association between DHEAS and juvenile characteristics may be due to the difference in time at which they were assessed. The presence or absence of secondary sexual characteristics may be reported differently by these populations and further validation of the PDS-A is needed. Despite these limitations, ABBY is the first study to demonstrate variation in the timing of adrenarche, specifically among migrants.

CONCLUSION

The age of adrenarche among first generation girls implies that the timing of adrenarche may be, in fact, plastic but mediated by environmental factors including, but not restricted to energetic status. Psychosocial factors may interact with BMI and influence the timing of adrenarche through the concept of biological sensitivity to context. This concept will be explored further in Chapter 6, the final discussion of my thesis.

Figure 30: Median Ages of Adrenarche among Sylheti, First Generation, Second Generation and white British girls.



Each horizontal line represents a segment of the life course for each migration group which are ordered in increasing order of the migration scale. The black diamond represents the universally accepted age at adrenarche. The blue diamonds summarize the age at adrenarche for each migration group.

CHAPTER 4: PUBERTY AMONG BANGLADESHI AND BRITISH YOUTH

SUMMARY

Age at sexual maturity, typically marked by menarche, varies widely across human populations according to ethnicity and ecology, and within populations by socioeconomic and nutritional status. Discontinuity in ecological conditions during childhood may also be associated with the timing of menarche among migrants groups. For example, the recalled age at menarche differed among Bangladeshi migrants to the UK depending on the age at which they migrated (Núñez-de la Mora et al. 2007). Women who migrated as children reached menarche earlier than women who migrated as adults, suggesting that the developmental environment affects the pace of reproductive strategies. There is increasing evidence that that the timing of pubertal onset, as marked by breast and/or pubic hair development, may influence adult reproductive health outcomes such as susceptibility to conditions including breast cancer or PCOS (Ibáñez et al. 2000; Biro et al. 2003). Little is known about how juvenile development, puberty's precursor stage, affects reproductive development. My aim is twofold: 1) to test whether the chronological ages of different markers of puberty (thelarche, pubarche and menarche) differ across populations of Bangladeshi and British girls and 2) to compare the order and tempo of adrenarche with thelarche, pubarche and menarche across the same populations. Comparing the timing of adrenarche, thelarche, pubarche and menarche in the above ways between migration groups of girls growing up in different environmental conditions sheds light on how the developmental environment is associated with the patterns of pubertal development.

INTRODUCTION

Puberty marks the end of the juvenile period, yet puberty is a transition itself encompassing morphological, physiological and hormonal changes including menarche, thelarche (breast development), pubarche (pubic hair development) and growth in height and weight. Empirically, pubertal onset is marked by thelarche or pubarche, respectively measured by the secondary sex characteristics of breast and pubic hair development and mediated by the underlying increased production of sex steroids, including oestrogens and androgens. Menarche occurs relatively late in the pubertal transition but is a strong marker that central puberty, an active hypothalamic-pituitary-gonadal (HPG) axis, is in place. Thelarche, pubarche and menarche are collectively referred to as gonadarche because together they reflect the activation of the HPG axis. Between the early 1800s and mid-1900s, age at menarche underwent a secular decline from ages 15 –17 years to about ages 13 – 13.5 years in Western populations (Kaplowitz 2006). Since 1960, it appears that age at menarche has stabilized (ibid). Despite this, age at pubertal onset continues to appear earlier and, in the last 40 years, mean pubertal onset has occurred 0.5 – 1.0 year earlier among American girls (ibid). It is debatable whether the first appearance of these secondary sex characteristics reflects the true onset of puberty (as reviewed in Chapter 1).

Adrenarche, which begins the juvenile period, is argued to be a separate process from puberty (Sklar et al. 1980) because it reflects the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Adrenarche is heralded by a rise in DHEAS and may be linked to androgen-mediated changes in the sebaceous glands resulting in body odour, oily skin and sexual hair growth (Campbell 2006). The timing of adrenarche appears to occur within a relatively narrow two year window across migration groups, between the ages of 6—8 years (Sizonenko and Paunier 1975, Korth-Schutz et al. 1976, Reiter et al. 1977, Goñez et al. 1993). However, there is some evidence that in extreme cases – being born small for

gestational age (Ibáñez et al. 2000), or experiencing a country-level change in ecology, as observed in first generation Bangladeshi migrants to the UK (see Chapter 3) — premature adrenarche may occur. Few studies have examined the timing of adrenarche in relation to pubertal onset (Sizonenko and Paunier 1975; Sklar et al. 1980; Biro et al. 2003); almost none have adequately looked at the interval between adrenarche and menarche across populations because few have recruited girls young enough to actually capture the onset of adrenarche. Despite this, recent studies suggest that adrenarche may fine-tune the timing of pubertal development (Remer et al. 2010).

The pattern of pubertal development can be assessed by different measures of time including chronology (age), order of events (pathways) and tempo (interval between each stage) (Biro 2010). It is important to separate out these measures of time when assessing the relationship between adrenarche and gonadarche and to test whether populations differ in their pattern of development.

The Early Environment and Reproductive Trajectories

Children appear to be able to adjust pubertal development adaptively to match local conditions (Belsky et al. 1991) as illustrated by a series of adoption studies. Adoption studies, which complement migrant studies in that they can compare genetic and environmental factors, have demonstrated that adopted girls enter puberty earlier than girls living with their biological parents. One classic study found that Indian girls who were adopted into Swedish families reach menarche earlier than non-adopted girls living in India and Sweden (Proos et al. 1991). The age at menarche was correlated with age at adoption and related to the length of residence in the pre-adoptive environment and/or time since moving to the post-adoptive environment. The Indian girls who arrived in Sweden and were adopted between ages 3 – 7 years reached menarche earlier than girls that were adopted

before age three and after age seven (ibid). Menarche occurred earliest if adoption occurred during a critical window, in this case early childhood. Another adoption study of Southeast Asian adoptees to Denmark, conducted with pre-pubertal girls aged 5 – 9 years, found that adopted girls showed signs of increased pituitary and gonadal hormonal levels despite having no sign of secondary sex characteristics when compared to Danish controls (Teilmann et al. 2007). Not only does the latter study demonstrate activation of the HPO axis before there are any physical signs of secondary sex characteristics, it also shows an earlier onset of puberty among girls whose developmental environment changed during childhood. Interestingly, there were no differences in BMI between adoptees and controls. The authors suggest that factors other than body fat are likely to explain an earlier sexual maturation in adopted girls. Low birth weight, immune activation and psychosocial stress are all contenders, but more research is needed to confirm such mechanisms.

There is strong evidence that the timing of puberty has lasting effects into adulthood. Apter and Vihko (1989) studied pubertal onset among girls and followed up with the participants 30 years later. They found that hormonal characteristics observed during puberty were maintained into adulthood; women with an early menarche had higher serum oestradiol concentrations during the follicular phase of the menstrual cycle than women with a later menarche. Núñez-de la Mora et al. (2007) found significant differences in self-reported ages at menarche in Bangladeshi migrant groups to the UK. Women who migrated as children reached menarche at an earlier age than Sylheti women living in Bangladesh, women who migrated as adults and a comparative group of white women. Women who migrated as children had higher levels of salivary progesterone than women who migrated as adults. Given these findings from Bangladeshi migrants, in the study presented here, I tested for migration group differences in the timing of pubertal development according to the appearance of secondary sexual characteristics and menarche among girls aged 5-16 years.

HYPOTHESES AND PREDICTIONS

I compare the timing of puberty according to ethnicity, ecology and migration previously outlined in Chapter 1. If puberty differed by ethnicity, then all Bangladeshi girls would reach adrenarche at a different age than white British girls. If puberty differed by migration scale, then girls would increasingly reach puberty earlier with increasing individual/ancestral generations lived in the UK. If puberty differed by country-level ecology, then Sylheti girls would reach puberty later than all girls living in the UK. If puberty differed by discontinuity in ecology, first generation girls would reach puberty earlier than all girls. I tested the following hypothesis and predictions among Bangladeshi and British girls which subscribes to the ecological model of development.

Hypothesis: The overarching hypothesis is that puberty as measured by thelarche, pubarche, menarche, oestrogen and growth will differ according to ecology rather than ethnicity or migration scale. First generation British-Bangladeshi girls who spend part of their lives in London will reach thelarche, pubarche and menarche at an earlier age, will grow faster and show higher oestrogens than Bangladeshi girls who live in Sylhet and second generation and white British girls who live in the UK.

- Prediction A: Among girls older than age 9.5 years, age-specific anthropometric indicators (height, weight, BMI and waist circumference) will increase across the migration groups in the following order: Sylheti, second generation British-Bangladeshi and white British girls, and first generation British-Bangladeshi
- Prediction B: Age at pubertal onset (assessed by either thelarche and/or pubarche) will decrease across the migration groups in the following order:

Sylheti, second generation British-Bangladeshi and white British girls, first generation British-Bangladeshi

- Prediction C: Age at menarche will decrease across the migration groups in the following order: Sylheti, second generation British-Bangladeshi and white British girls, and first generation British-Bangladeshi
- Prediction D: Age-specific oestrogen levels will increase across migration groups in the following order: Sylheti, second generation British-Bangladeshi and white British girls, first generation British-Bangladeshi

In addition to the predictions in relation to ecology, the data from ABBY can also test the hypothesis that adrenarche is not a pathway through puberty but a stage that precedes all other stages.

- Prediction E: All girls will progress through puberty in the following order: adrenarche, followed by either thelarche or pubarche, then menarche.
- Prediction F: The tempo between adrenarche and menarche will be the same across migration groups, but both stages will occur earlier among girls living in the UK.

METHODS

PARTICIPANTS

Out of a total of 488 girls who were enrolled into the study, 415 girls (Sylheti= 168, first generation = 40, second generation = 159, white British= 48) provided complete pubertal stage data and from these, 326 (Sylheti= 155, first generation = 30, second generation = 112, white British= 29) provided urine samples that were analysed for oestrogen and oestrogen metabolites.

MEASUREMENTS

QUESTIONNAIRE AND PUBERTAL DEVELOPMENT SCALE

Study data were collected using a standardised form for each enrolled subject. The form elicited information on the girl's age, migration history, ethnicity, socioeconomic status, family composition, presence or absence of menses, age at menarche, and pubertal staging. Pubertal staging was assessed via self-report using a modified version of the Pubertal Development Scale (PDS). More details pertaining to the questionnaire can be found in Chapter 2.

URINARY OESTROGENS AND OESTROGEN METABOLITES

Details of urine collection and analysis are included in Chapter 2. In summary, a total of 326 urine samples were analysed for 15 oestrogen and oestrogen metabolites at the National Cancer Institute's Laboratory of Proteomics and Analytical Technologies (LPAT) using liquid chromatography- tandem mass spectrometry (LC-MS/MS) according to previously published methods (Xu et al. 2005). Overall coefficients of variation for quality control samples were less than 5%. Aliquots from the same urinary samples (including QC) were

also analysed for creatinine using an enzymatic colorimetric assay (PPD ©); all total laboratory CVs were below 2%.

All 15 oestrogen/oestrogen metabolites were adjusted for creatinine and summed to create a total measure of oestrogen exposure which I use to reflect circulating levels of oestrogen (Shi et al. 2010). Urinary oestrogens can be measured as independent oestrogens or grouped together according to metabolic pathways in order to explore associations of oestrogen metabolism and specific health outcomes (Furhman et al 2012). In this study, the sum of urinary oestrogen/oestrogen metabolites is used as a marker of circulating oestrogen in girls whose ovarian oestrogen production is just beginning.

SALIVARY DEHYDROEPIANDROSTERONE-SULFATE (DHEAS)

The details of saliva collection and DHEAS analyses have been presented previously in Chapters 2 and 3. In summary, saliva was collected using gum base as a stimulant and stored frozen at -20 °C until defrosted and analysed using an ELISA at the Durham Endocrinology and Ecology Laboratory. Adrenarche status was defined by DHEAS levels above 400 pg/ml.

Anthropometry

The methods by which anthropometrics were measured have been described previously in Chapter 2. In summary, measurements included: height, weight and waist circumference. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters.

Statistical Analyses

Anthropometrics

Multivariate linear regressions and ANOVA were used to evaluate differences in anthropometric measurements by migration group. Height, weight, BMI and waist circumference were compared among all girls more than aged 9.5 years to compare size after the onset of juvenility and during puberty. This was also the median age of all girls in the sample and the age when 90% of the girls in our study had reached adrenarche.

Height-for-age and weight-for-age z-scores were calculated and categorised into quartiles. Age-specific quartiles for waist circumference were determined by placing each girl into age-appropriate categories. BMI z-scores were also calculated for each girl and compared to UK growth references to determine the percentage of girls who would be clinically underweight, normal weight, overweight, or obese (Cole et al. 1998). It is standard to determine wasting or obesity by marking girls' BMI ± two standard deviations from the mean (upper and lower 2.5%). By classifying girls this way the majority of girls (64%) were classified as normal weight.

PUBERTAL DEVELOPMENT

The timing, order and tempo of pubertal development were compared across migration groups both descriptively and quantitatively. Binary variables for thelarche and pubarche were based on a girl reporting having reached Tanner stage 2 or greater for breast or pubic hair development, respectively. The proportions of girls in each migration group having reached thelarche and pubarche were compared across migration groups by age-specific quartiles.

Estimates for median ages at thelarche, pubarche and menarche were modelled using a Weibull regression model in STATA, described by Royston (2001). This model accounts for double censoring in the data; some of the girls had not yet reached puberty at their current age (right-censored) or reached puberty at some unknown age in the past (left-censored). This method has the advantage over the status quo method which uses current age and the absence or presence of the pubertal stage (Marshall and Tanner 1986). This allowed me to calculate and plot the entire distribution of ages at onset for each pubertal stage (in particular, the median age at onset). Goodness-of-fit of the Weibull regression models was assessed by graphical inspection of the of the Weibull survival curves to the non-parametric survival estimate akin to Kaplan-Meier (Appendix 6).

The ages of thelarche, pubarche and menarche were also modelled adjusting for anthropometric z-scores. The associations of anthropometrics with thelarche, pubarche and menarche were stratified by migration group.

The order of pubertal stages was assessed by comparing the median age of each stage within each migration group. The tempo of pubertal development was calculated as the difference in median ages at onset of each stage and the intervals were compared across migration groups by chi-square tests. The tempos of juvenile, pubertal and sexual development were

calculated by comparing the median ages at adrenarche (Chapter 3) with the median ages of thelarche, pubarche and menarche across migration groups.

Total Oestrogen and Oestrogen Metabolites

A measure of total urinary oestrogen and oestrogen metabolites is described by the variable, oestrogen. Concentrations of oestrogen were log transformed to reduce positive skewness and were compared using ANOVA and multivariate linear regression models, where log(oestrogen) was the dependent variable, migration scale the independent variable and covariates were entered in a forward step-wise fashion in the following order: age, BMI, height and weight. Models were developed for all girls and restricted to pre-menarche girls because variability in oestrogen during the menstrual cycle may complicate comparisons between migration groups. Models to detect differences in age at thelarche, pubarche and menarche by migration scale were run, both unadjusted and adjusted for oestrogen.

RESULTS

ANTHROPOMETRICS

Among girls older than age 9.5 years, when puberty is most likely to begin, weight, BMI and waist circumference all increased according to migration scale ($p<0.01$) (Table 10). While migrant groups were taller than Sylhetis, white British girls were not ($p=0.07$).

BREAST DEVELOPMENT AND PUBIC HAIR GROWTH

Figure 31 summarises the proportion of breast and pubic hair development by age quartiles and migration group. Even at the youngest age quartile (<7.5 years), a small proportion of girls showed breast development and the proportion increased incrementally with age. Among white British girls, there is a noticeably small increase in breast development between the second and third age quartiles. A small proportion of Sylheti and second generation British-Bangladeshi girls reported pubic hair development in the youngest age quartile and the proportion increased incrementally with age. British girls reported pubic hair development after age 7.5 years and this proportion substantially increased to 80% after age 9. On average, pubic hair appears two years after breasts. Pubarche increased with migration scale, but there was a delay in onset in first generation migrants.

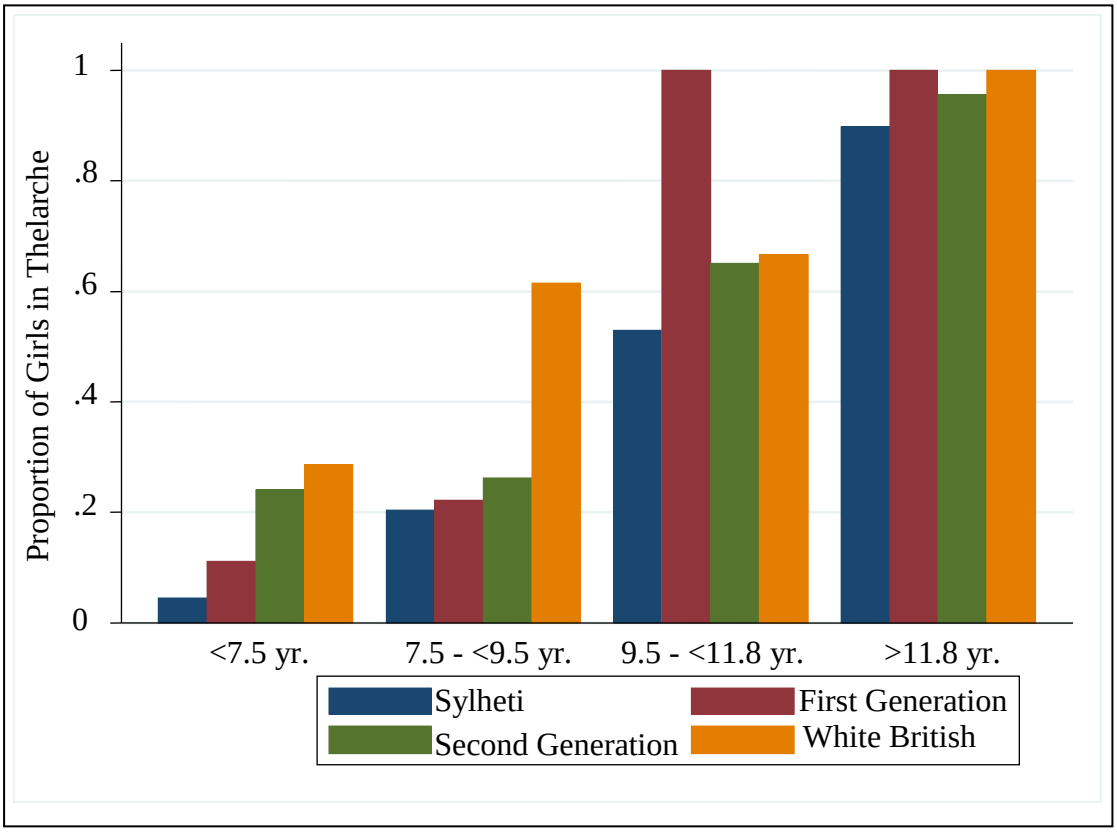
Table 10: Summary of Sample and Anthropometrics Comparing Sylheti, First Generation, Second Generation and White British Girls Older than Age 9.5 Years*

	Bangladeshi		First Gen.		Second Gen.		White British		ANOVA P-value
N	81		23		95		28		
Age (years)	12.6	(1.9)	13.4‡	(1.9)	12.2	(1.7)	11.7	(1.6)	<0.01
Height (cm)	144.0	(11.3)	148.7	(7.7)	147.9	(9.8)	144.9	(11.8)	0.07
Weight (kg)	37.8†	(10.6)	47.1	(10.2)	45.9	(12.3)	44.9	(13.8)	<0.01
Body Mass Index (kg/m²)	17.9†	(3.1)	21.2	(3.6)	20.5	(3.6)	20.9	(4.1)	<0.01
Waist Circumference (cm)	58.3†	(6.9)	65.8	(8.6)	67.8	(9.3)	68.1	(9.1)	<0.01
BMI Z-Scores	-0.41†	(1.13)	0.61	(1.02)	0.69	(1.08)	0.99	(1.13)	<0.01
UK Clinical Nutritional Status									
Underweight	24%		0%		6%		0%		
Normal weight	67%		67%		60%		60%		
Overweight	7%		29%		27%		19%		
Obese	3%		5%		7%		22%		

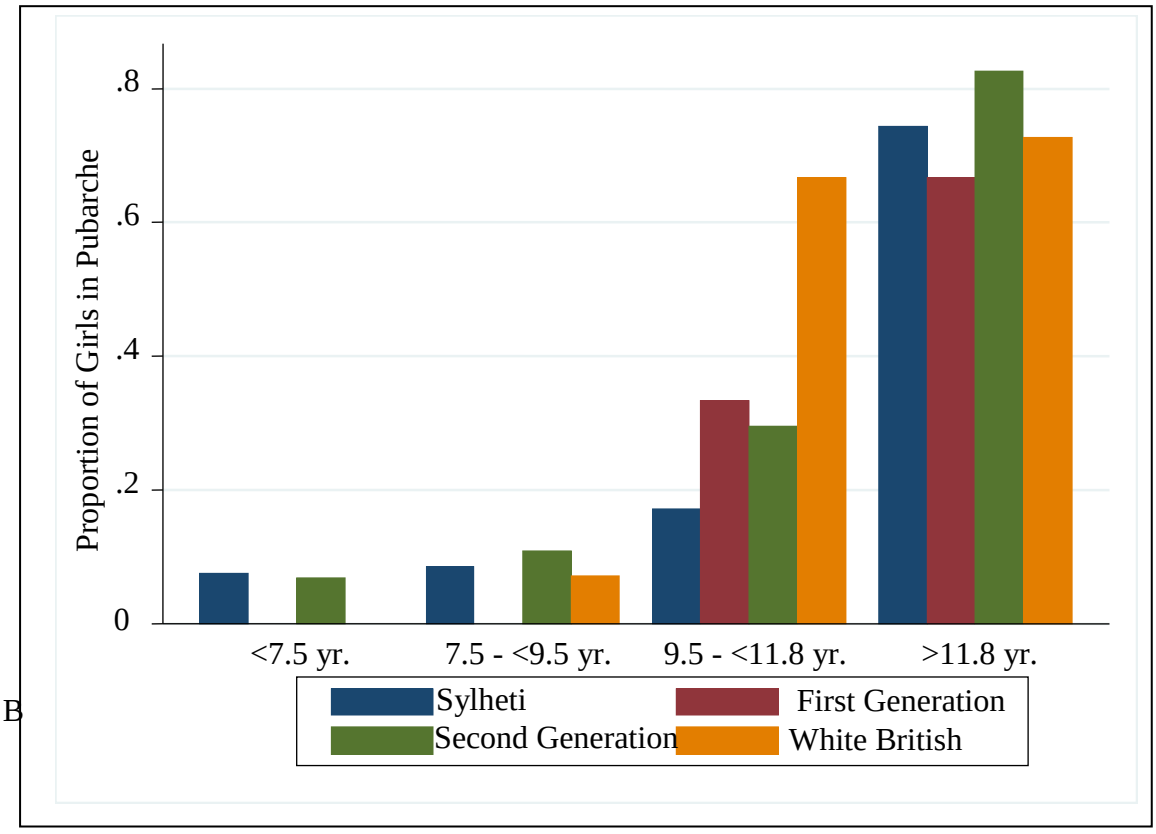
*Values given as mean (standard deviation)
†Significantly different from all groups living in the UK
‡ significantly different from second generation and white British

Figure 31: Comparison of the Proportion of Girls with Breast (A) and/or Pubic Hair Development (B) at Tanner Stage 2 or Greater by Age Quartile and Migration Group.

A



B



PUBERTAL TIMING, ORDER AND TEMPO

MEDIAN AGES OF THELARCHE, PUBARCHE AND MENARCHE AND THEIR ASSOCIATIONS WITH ANTHROPOMETRICS

Thelarche and pubarche occurred earlier with increasing individual/ancestral generations lived in the UK (Table 11; thelarche $p < 0.01$; pubarche $p < 0.01$), but menarche did not follow this trend (Table 11; p-trend= 0.7) Thelarche and pubarche appeared earliest among white British girls (median age 8.7 and 10.9 years, respectively). Menarche occurred earliest among first generation girls (median age 11.8 years).

Height, weight, waist circumference and BMI z-scores were associated with the timing of thelarche (Table 12). The overall trend for earlier thelarche with increasing migration scale, still remained even after adjustment for height (Table 12: HR: S = 1, BB1 = 1.6, BB2 = 1.3, WB = 2.5; p-trend=0.004). Differences among groups according to migration scale diminished when the model was adjusted by measures of adiposity (weight, waist circumference, BMI), but some differences between migration groups remained (Table 12).

Heights for age z-scores were associated with the timing of pubarche (12). The overall trend for earlier pubarche with increasing migration scale, while no longer significant, still remained even after adjustment for height (Table 12: HR: S = 1, BB1 = 0.7, BB2 = 1.2, WB = 1.6; p-trend=0.1). Differences among groups according to migration scale were slightly attenuated when the pubarche model was adjusted by weight, waist circumference and BMI, but differences between migration groups remained (Table 12).

Height for age was significantly associated with the timing of menarche (Table 12).

The associations between each anthropometric and the timing of thelarche and pubarche were explored independently of migration scale to identify which anthropometric was the dominant factor for each pubertal stage. Height was no longer a significant predictor of

thelarche when adjusted for weight (p=0.7), waist circumference (p=0.4) or BMI (0.1); BMI was no longer significant when adjusted for weight (p=0.1) or waist (p=0.2; weight was no longer significant when adjusted for waist circumference (p=0.3). Waist circumference remained a significant predictor of thelarche when adjusted for by height, weight or BMI ($P<0.001$). Height emerged as the dominant factor for pubarche as it remained significant after adjusting for weight, waist circumference and BMI.

Table 11: Comparison of Age at Onset of Thelarche, Pubarche and Menarche Among Migration Groups Using Weibull Regression Models for Parametric Survival Analysis

Age at Onset of Thelarche, Pubarche and Menarche										
		Thelarche			Pubarche			Menarche		
Migration group	n	Median	HR	(95% CI)	Median	HR	(95% CI)	Median	HR	(95% CI)
Sylheti	162	10.7	1.0		12.5	1.0		12.5	1.0	
First Gen.	40	9.2	2.0	(1.0- 4.1)	13.2	0.8	(0.4- 1.5)	11.8	1.8	(0.8- 4.0)
Second Gen.	159	9.6	1.6	(1.1- 2.4)	11.6	1.5	(1.0- 2.3)	12.1	1.4	(0.8-2.4)
White British	48	8.7	2.6	(1.5- 4.4)	10.9	2.0	(1.1- 3.7)	12.6	0.9	(0.4- 2.1)
Migration Scale Trend Test		<0.01			<0.01			0.70		

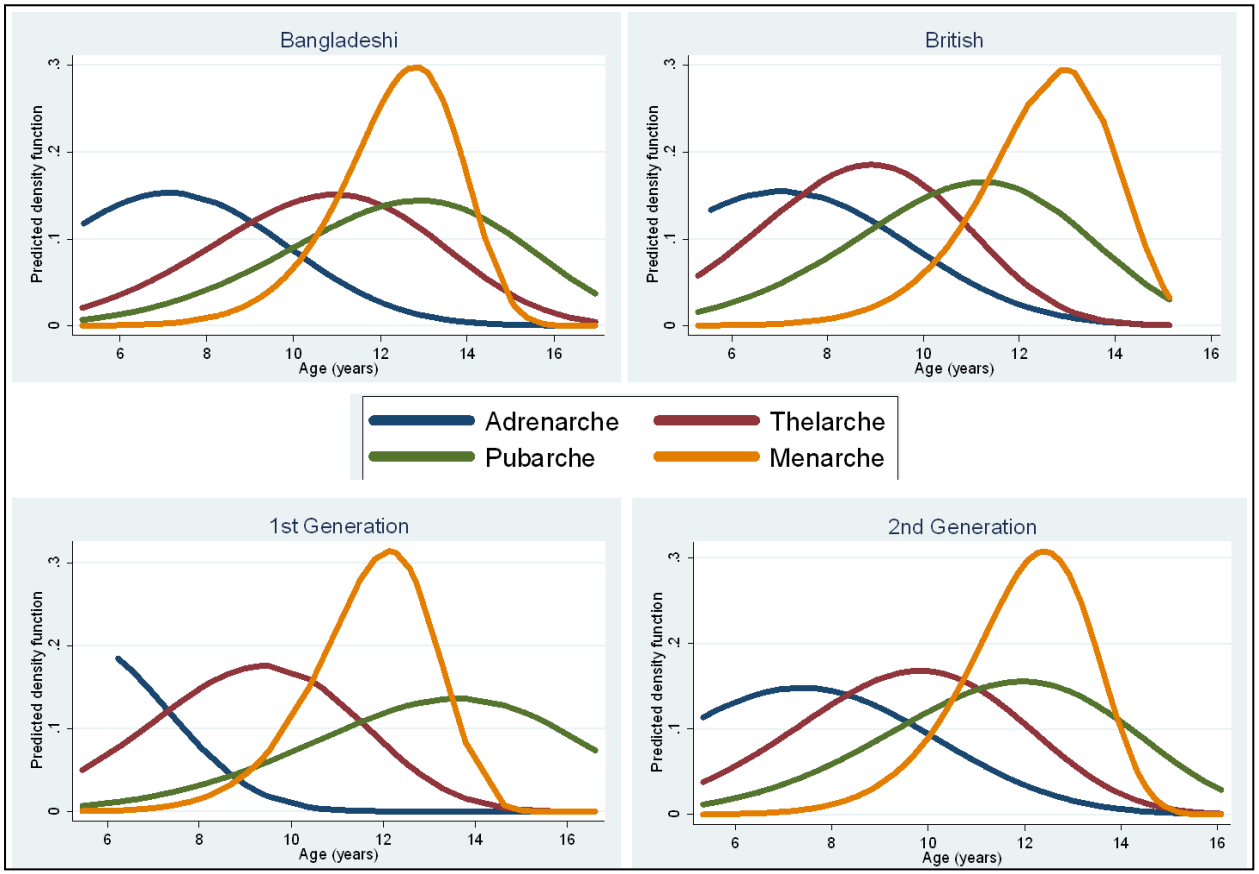
Table 12: Hazard Ratios of the Ages of Thelarche, Pubarche, and Menarche According to Anthropometric Quartiles and the Association of Anthropometrics on Age at Development Stratified by Migration Group using Weibull Regression Models for Parametric Survival Analysis.

Thelarche				HAZ	WAZ	Waist	BMI Z
	Anthropometric	Quartile 1		1.0	1.0	1.0	1.0
		Quartile 2		1.4	1.5	1.7	1.1
		Quartile 3		1.6	2.8	2.9	1.3
		Quartile 4		2.5	3.3	3.2	1.3
		p-trend		0.001	<0.001	<0.001	<0.001
	Migration group	Sylheti	1.0	1.0	1.0	1.0	1.00
		1st	2.0	1.6	1.3	1.3	1.4
		2nd	1.6	1.3	1.0	1.0	1.1
		WB	2.6	2.1	1.7	1.5	1.7
		p-trend	<0.01	<0.01	0.3	0.5	0.2
Pubarche							
	Anthropometric	Quartile 1		1.0	1.0	1.0	1.0
		Quartile 2		1.7	1.4	1.8	1.1
		Quartile 3		2.5	1.5	1.4	1.0
		Quartile 4		3.2	1.8	1.6	1.0
		p-trend		<0.01	0.05	0.3	0.86
	Migration group	Sylheti	1.0	1.0	1.0	1.0	1.0
		1st	0.8	0.7	0.6	0.8	0.7
		2nd	1.5	1.2	1.3	1.6	1.6
		WB	2	1.6	1.7	2.2	2.2
		p-trend	<0.001	0.1	0.05	<0.001	0.02
Menarche							
	Anthropometric						
		Quartile 1		1	1.0	1.0	1.0
		Quartile 2		1.6	2.3	2.3	1.2
		Quartile 3		2.9	2.0	1.3	1.1
		Quartile 4		3.2	2.6	1.6	1.2
		p-trend		<0.01	0.08	0.4	0.13
	Migration group	Sylheti	1.0	1.0	1.0	1.0	1.0
		1st	1.8	1.6	1.3	1.9	1.4
		2nd	1.4	1.0	0.9	1.3	1.0
		WB	0.9	0.7	0.6	0.9	0.6
		p-trend	0.7	0.5	0.3	1.0	0.23

Order and Tempo of Pubertal Development

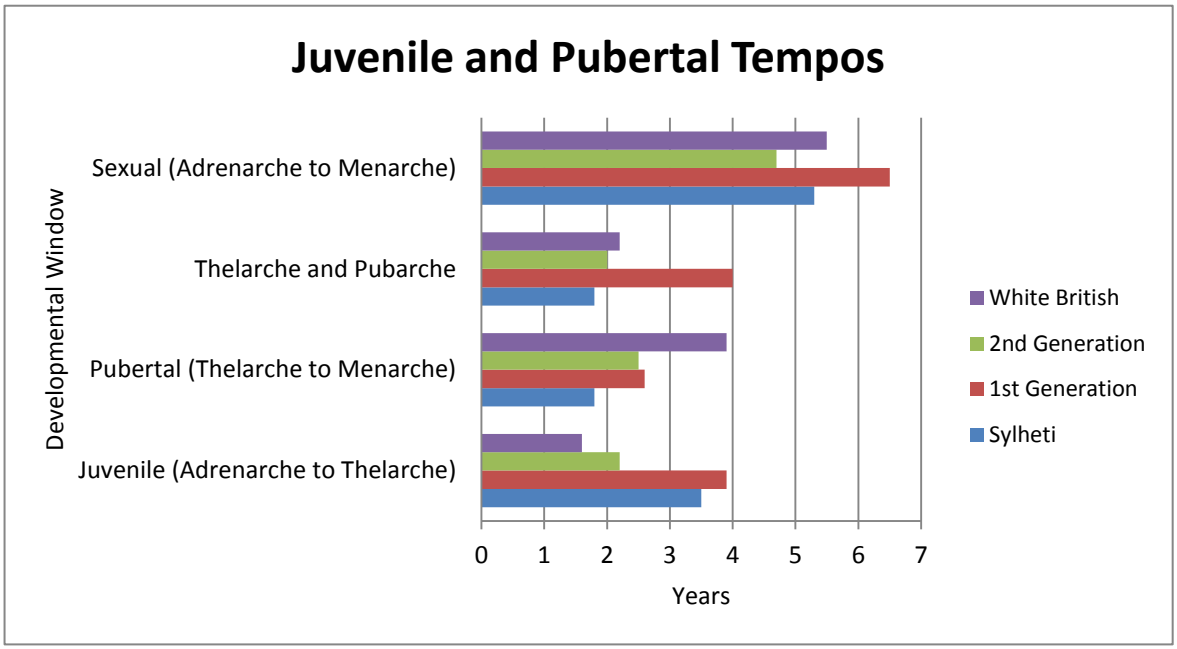
The order of sexual development, including the median ages of adrenarche from Chapter 3, was similar across the Sylhetis, second generation and white British girls and proceeded in the following order: adrenarche, thelarche, pubarche, and menarche (Figure 32). However, first generation girls reached pubarche after menarche. The intervals between the intermediate stages, specifically the stages between adrenarche and menarche, differed across Migration groups (Figure 33). Among the migration groups, the mean interval between adrenarche and menarche was longest for first generation girls (6.4 years) and shortest for second generation girls (4.7 years). The interval between thelarche and menarche was longest (3.9 years) in white British girls due to early breast development. The interval between adrenarche and pubarche was longer (5.5 years) in the Sylheti girls when compared to second generation and white British girls due to a later onset of pubarche. First generation British-Bangladeshi girls also had the earliest age at adrenarche and latest age at pubarche, so the interval between adrenarche and pubarche was 7.9 years.

Figure 32: Comparison of the Density Distribution of Ages at Adrenarche, Thelarche, Pubarche and Menarche in Each Migration Group



The order of sexual development was similar across groups and proceeded in the following order: adrenarche (blue), thelarche (red), pubarche (green) and menarche (yellow). However, first generation girls reached pubarche after menarche. The graphs also illustrate that thelarche (red) and pubarche (green) shift to the right across the migration groups, meaning they occur earlier with increasing individual/ancestral generations in the UK.

Figure 33: Juvenile and Pubertal Tempos Among Migration Groups



The tempos between stages of sexual development differ by period and group. The tempo of juvenility is more rapid in the UK. The interval (in years) between adrenarche and thelarche is as follows: Sylheti = 3.5, first generation = 3.9, second generation = 2.2, white British = 1.6. The tempo of puberty is slower in the UK; and, the interval between thelarche and menarche is as follows: Sylheti= 1.8, first generation = 2.6, second generation = 2.5, white British = 3.9. The interval between thelarche and pubarche is similar among all groups [Sylheti = 1.8, second generation = 2, white British = 2.2], except longer in the first generation girls [4]. Sexual development from adrenarche to menarche is also relatively similar among all girls [Sylheti = 5.3, second generation = 4.7 and white British = 5.5], with the exception of slower tempo among the first generation girls = 6.5.

Comparison of Oestrogens by Migration Group

Overall, there is no evidence that the urinary concentration of the sum of oestrogens and oestrogen metabolites differs significantly across migration groups. Table 13 summarises oestrogenic variation by migration group across age quartiles. The results from linear

regressions that compared the relationship between oestrogen and migration scale, age and age–specific BMI separately suggested that total oestrogen increased with age (β1=0.25; p<0.01), but there was no relationship with migration scale (β1= -0.04, p= 0.48) or BMI (β1=0.00; p=0.96). Oestrogen decreased significantly with increasing individual/ancestral generations lived in the UK when adjusted for both age and BMI (β1= -0.13; p= 0.03), but was not statistically significant after adjusting for age alone (β1= -0.09; p=0.09). Oestrogen was compared between each migration group adjusted for both age, and age and BMI, respectively. Both second generation and white British girls had lower oestrogen than Sylhetis when adjusting for age and BMI, but this difference was only statistically significant among the white British girls (β1= -0.52; p= 0.02). First generation girls had higher oestrogen than Sylhetis, but this was not statistically significant (β1= 0.19; p=0.48).

Table 13: Comparison of Total Oestrogen/ Oestrogen Metabolites (pmol per mg creatinine) Among Sylheti, First Generation, Second Generation, White British girls stratified by Age Quartiles using ANOVA*

	Sylheti		First Generation		Second Generation		White British		ANOVA p-value
N	155		30		112		29		
<7.5 yr.	1.9	(0.7)	2.5	(0.9)	1.4	(0.9)	1.6	(1.0)	0.052
7.5 - <9.5 yr.	1.9	(0.8)	2.3	(0.9)	1.9	(0.7)	1.5	(1.2)	0.259
9.5- <11.8yr.	3.0	(0.8)	1.9	(1.0)	3.0	(1.0)	2.9	(0.7)	0.222
>11.8 yr.	3.8	(0.9)	3.4	(1.0)	3.8	(0.9)	3.4	(1.2)	0.804

*Values were log transformed and reported as means and SD.

DISCUSSION

The ABBY Project was envisioned to compare the timing of adrenarche with thelarche, pubarche and menarche by both physical and hormonal markers across populations that differ by ethnicity, ecology and migration scale. Using a model of a migrant Bangladeshi population, this study found that both white British and migrant groups living in the UK progressed through puberty earlier and slower than their Sylheti counterparts living in Bangladesh. Age at thelarche was earliest among white British girls and latest among Sylheti girls. Energy balance, as measured by BMI and waist, attenuated some but not all of the differences in pubertal development. With the exception of the first generation migrants, median ages at adrenarche and menarche were similar across migration groups. The tempo of juvenile development is faster among second generation and white British girls than Sylheti and first generation girls. Once the juvenile period is over, pubertal tempo is slower among all girls living in the UK compared to girls living in Bangladesh. While pubertal development occurs earlier with increasing individual/ancestral generations lived in the UK, oestrogen levels did not follow this migration scale trend. Thus, the pattern of pubertal development as marked by secondary sex characteristics differs from the pattern of pubertal hormones, namely oestrogens and oestrogen metabolites. It appears that the timing of thelarche and pubarche are sensitive to environmental conditions. Lifestyle factors such as diet, disease or psychosocial aspects that differ between Bangladesh and the UK may alter the timing of pubertal onset. The most dramatic differences in age at adrenarche and menarche were observed in the first generation girls and these findings suggest that discontinuity in ecology is associated with their timing.

The data presented here suggest that the age of pubertal onset declines with increasing individual/ancestral generations lived in the UK. Compared with girls living in Bangladesh,

thelarche and pubarche occurred at increasingly earlier ages among all girls living in London. This study has found that first generation, second generation and white British girls develop breasts 1.5, 1.1 and 2.0 years earlier than Sylheti girls, respectively. The relationship between earlier thelarche and migration scale was partially accounted for by higher BMI among those migration groups living in the UK, suggesting that local-level lifestyle changes across generations impact the timing of breast development. Increased body fat, possibly mediated by leptin, has been previously linked with an earlier pubertal onset (l'Allemand et al. 2002). However, not all the variation between migration groups was explained by measures of adiposity. Adoption studies have similarly suggested that other factors may affect the timing of puberty (Teilmann et al. 2007), especially among girls who experience a change in environment early in life (Proos et al. 1991).

The ages at thelarche (population median age range: 8.7 – 10.7 years) are markedly earlier than the average age of breast stage 2 (age 11.15 years) first reported for British girls in the classic Marshall and Tanner study (1969). My findings are consistent with more recent studies that show breast development occurring between ages 9 – 11 years across human populations (Herman-Giddens et al. 1997; Huen et al. 1997; Mul et al. 2001; Mahachoklertwattana et al. 2002; Wu et al. 2002; Juul et al. 2006). In my study, white British girls reached thelarche particularly early (age 8.7 years) compared to all Bangladeshi girls and girls in other studies (Chapter 1: Table 2). One other study, among American girls, reported thelarche to begin at age 8.8 years among African-American girls (Herman-Giddens et al. 1997), but their study has been criticised for being biased to clinical conditions rather than a representative sample of healthy girls.

The data do not statistically support the prediction that age at menarche varies across migration groups. However, age at menarche still differed descriptively between migration groups according to ecology. The median age at menarche was similar between Sylheti and

white British girls, but Sylhetis and white British reached menarche approximately eight and five months later than first generation and second generation girls, respectively. A difference in age at menarche of 5 – 8 months between populations may or may not be biologically significant.

The ages at menarche among girls in my study follow the same pattern by migration history but are earlier than recalled ages of menarche assessed among women aged 18 – 39 years in the previous study conducted by Núñez-de la Mora and colleagues (2007). Mean ages at menarche for the women in the previous study were: Sylheti =13.2 ± 0.2 yr, child migrants= 12.2 ± 0.2 yr, adult migrants = 13.0 ± 0.2 yr and white British = 13.1 ± 0.2 yr (Núñez-de la Mora et al. 2007). The difference in overall ages between my study and that of Núñez-de la Mora et al. may reflect differences in the methods used or there may be a cohort effect. Just as a secular decline in the age at menarche has been observed in Western countries, the age at menarche may also be declining among middle class Sylheti females. Other South Asian countries have observed differences in the age at menarche between socioeconomic groups. For example, poor Indian girls reach menarche at a median age 14 while economically more advantaged Indians reach menarche at age 12 (Roberts et al. 1977). Thus, the earlier age at menarche observed in the current study may be due to the changing landscape in Sylhet due to the influx of financial remittances from abroad and the increasing urbanisation taking place within the region (Sharma and Zaman 2009).

The earliest age of menarche among the first generation girls mirrors the finding among child migrants in the Núñez-de la Mora et al. (2007) study and points to the influence of early life environment on the timing of sexual maturation. Menarche for first generation girls occurred at age 11.8 years in my study, which was the earliest age among all the populations studied in this and Nunez- de la Mora et al.'s study. Other studies conducted with adopted children from India into Sweden also found that menarche was earliest among those girls who were

adopted at earlier ages (Proos et al. 1991). Together, these findings point to a change in environment during a critical time period altering age at menarche.

ABBY results do not support the prediction that oestrogen varies by ecology. The relationship among pubertal timing, migration groups and oestrogen levels in the current study is puzzling. One major strength of this study compared to the only other study that compared juvenile levels of urinary oestrogens is the specificity and sensitivity of the LC-MS/MS assay. All 15 oestrogens and oestrogen metabolites were detectable in all our samples with very low within and between batch CVs; whereas, previous studies were only able to compare five oestrogen metabolites because several metabolites were below the limit of detection (Shi et al. 2010). Before controlling for age, oestrogen increased with the prevalence of breast and of pubic hair development and menarche (Chapter 2). In survival models, oestrogen was positively associated with both pubarche and menarche; however, there was no relationship with thelarche (data not shown). This apparent contradiction may be due to over-reporting of breast development among girls. However, Radfar (1976) previously found differences in oestrogen levels by age even though all girls were in breast stage 1, suggesting that there is substantial variation in oestrogen production regardless of breast development. The first generation girls had the earliest menarche and highest oestrogen levels. On the other hand, oestrogen was relatively high among Sylheti girls even though they had the latest puberty and lowest BMI. This is contrary to what was expected because puberty should manifest physically as a result of increased oestrogen production. Likewise, oestrogen should be lower among thinner girls because, in fatter girls, there is more oestrogen due to the peripheral conversion of androgens into oestrogens in fat tissue (Ahmed et al. 1999, Jasik and Lustig 2008, Shi et al. 2010).

The ABBY findings support the theoretical model of sexual development (Chapter 1) which states that adrenarche is not a pathway through puberty but a stage before pubertal onset. At

the population level, the onset of events occurred in the following order: adrenarche, thelarche, pubarche and then menarche. There were some exceptions: 10% of girls reported pubarche before reaching adrenarche, 7% of girls reached pubarche before thelarche, and 9% reached menarche before pubarche. The ABBY findings suggest that most girls reach adrenarche first and then progress through thelarche. Pubarche followed thelarche by two years in all migration groups except the first generation migrants. Pubarche and menarche then occur more synchronously. Biro et al. (2003) have previously distinguished between girls that progress through puberty along the thelarche pathway as opposed to what they refer to as the adrenarche pathway. Because most girls reached thelarche before pubarche, my study supports other studies that define pubertal onset by breast development. However, I disagree with those studies that dismiss using pubic hair as a marker of puberty. Pubarche was relatively synchronous with menarche among Sylheti and second generation girls and even occurred after menarche among first generation girls. This suggests that the order of pubertal events may differ between Bangladeshi and British populations at large, and that there may be phylogenetic differences in the skin's sensitivity to androgens between these ethnicities.

The data suggest that the tempo of sexual development differs by migration group. The tempo from the juvenile period to age at sexual maturity (menarche) was similar between Sylheti and white British migration groups but longer among first generation and shorter among second generation groups. The tempo through juvenile development (the interval between adrenarche and thelarche) was faster among girls born in the UK (second generation and white British) compared to girls born in Bangladesh (Sylheti and first generation). This suggests that the foetal or early childhood environment may be an important factor in setting the tempo of juvenile development. It is important to note that while the tempo of juvenility is similar between Sylhetis and first generation migrants, the first generation migrants

reached both adrenarche and thelarche earlier than Sylhetis suggesting that the postnatal childhood period before adrenarche is also a period of plasticity. The interval between adrenarche and thelarche was shortest among white British, suggesting that white British girls progressed from adrenarche to pubertal onset at a faster tempo. The interval between thelarche and menarche was longest among the white British, indicating that they have the slowest tempo from pubertal onset to menarche.

It is an apparent contradiction that the first generation girls had the earliest adrenarche but latest pubarche considering that androgens mediate sexual hair growth. The sensitivity of the skin to androgens may be an important factor in assessing pubic hair stage.

It appears that the timing of thelarche and pubarche is more plastic than that for adrenarche and menarche in these populations. That the timing of these endpoints was different among first generation British-Bangladeshis suggests that not only is it the early developmental environment that is associated with pubertal timing but that a change in environment may cause a more exaggerated response in pubertal timing.

LIMITATIONS OF THE STUDY

There are methodological issues that need to be considered when interpreting the findings reported here. There are limitations to using self-reported pubertal staging. In an earlier study, concordance between a physical exam conducted by a physician and the Pubertal Development Scale (PDS) was only .24 (Brooks-Gunn et al. 1987). Another study compared the PDS, separated into a gonadal (breast, somatic growth and menses) and adrenal score (pubic hair and skin changes), with both a physical exam and hormone measures. The study found that the PDS gonadal score was more reliably related to hormones than the physical exam, but the adrenal score was less predictive of girls' DHEA levels (Shirtcliff et al. 2009). I adapted the PDS to include more detailed questions related to adrenal production than was

tested by Shirtcliff and colleagues and this may enhance the ability of the PDS-A to reflect androgen levels.

Prior validation studies also found age, fatness and ethnicity to affect the correlations between the PDS and physical exam/hormone measures (Shirtcliff et al 2009, Raman et al 2009, Biro et al 2003 and 2010). Adolescents overestimated pubertal maturation when they were at lower stages of development relative to their peers and underestimated development when they were at higher stages than their peers (Shirtcliff et al. 2009). Another study found that self-assessment of Tanner staging among overweight children did not accurately assess their pubertal development when compared to a hormone-derived pubertal assessment method (Raman et al. 2009). Other studies demonstrate that fat tissue can be mistaken for breast tissue, especially in overweight girls, and they suggest using the Garn-Falkner system for areola stages or breast palpation (Biro et al. 2003; Biro et al. 2010). The latter can be used to minimize confounding of breast development by adipose tissue. Because my research was conducted in schools, physical examination was not feasible. In addition, only 5% of Bangladeshi girls in this study was clinically obese, compared to 22% of white British girls.

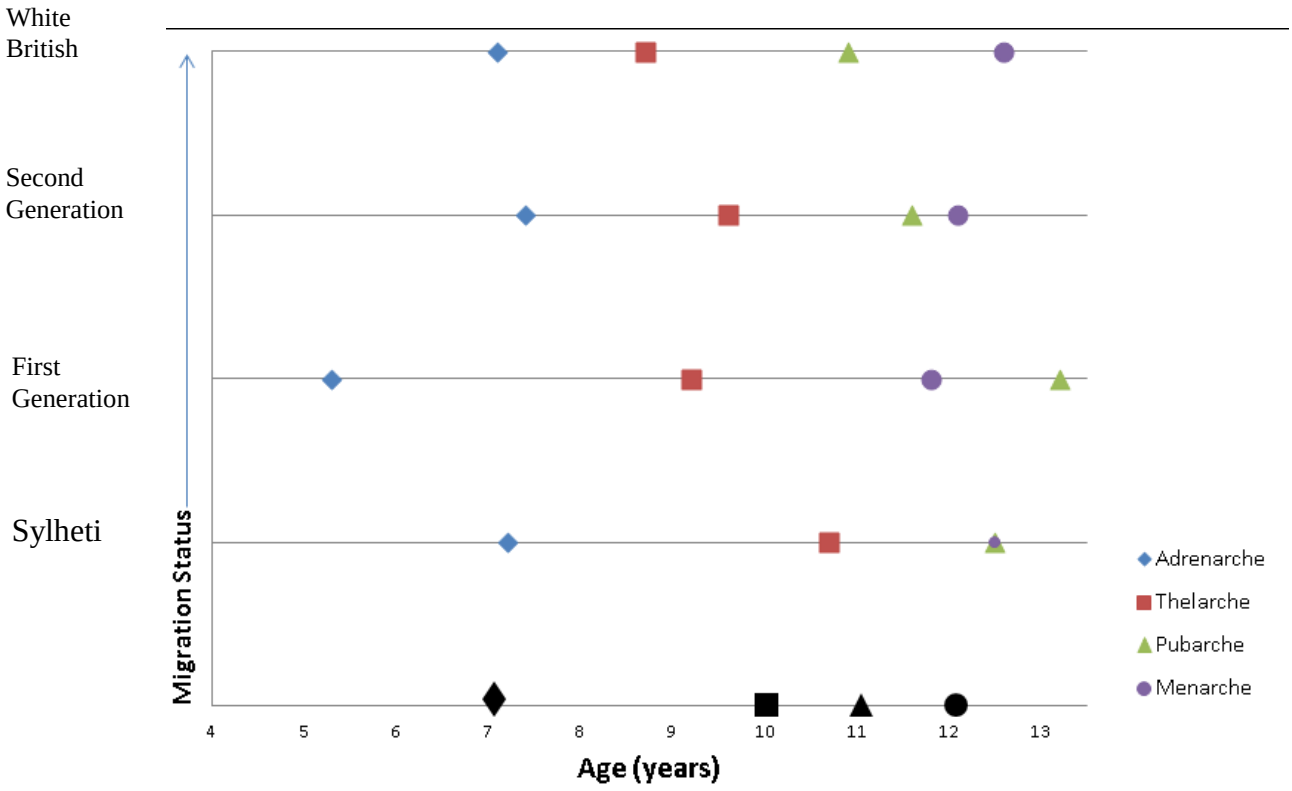
It has also been demonstrated that white adolescents overestimate their pubertal stage more often than non-Caucasian adolescents (Shirtcliff et al. 2009). Similarly, Bangladeshi girls may have been less likely to report breast development due to cultural perspectives of modesty. Because of this cultural perspective, girls were given the option to fill out the PDS-A questionnaire themselves, and from fieldwork experience this provided adequate privacy to help alleviate shyness. It was beyond the scope of ABBY to validate the PDS-A by visual inspection; using the PDS-A was the most feasible option in a field setting. We have internal validation of the PDS-A because oestrogen levels were higher among girls that reported having secondary sex characteristics compared to girls who did not (Chapter 2).

The comparison of age at menarche across populations is limited by the small sample size in both the white British and first generation migration groups. Only eight white British girls reported having reached menarche and so the age at menarche is determined by a small number of girls. Despite calculating median age at menarche using a survival model, I cannot confidently conclude that the age at menarche found in our study is representative of most white British girls growing up in East London. The first generation group, among whom are some of the most striking findings, are also a very small sample and so the differences found in this population may be larger than future replication studies. It is also possible that there is a self-selection bias among those Bangladeshis who migrate to the UK in that they differ from the Bangladeshis who remain in Bangladesh by socioeconomic, health, immunological or psychosocial factors.

CONCLUSION

This study supports the model that sexual development starts with adrenarche and then proceeds from thelarche to pubarche to menarche. The age at pubertal onset declined with increasing individual/ancestral generations lived in the UK. Girls who grew up in the UK progressed through the juvenile period faster and through puberty slower than girls who grew up in Sylhet. Age at menarche uniformly declined about one year compared to women from the same populations who reached puberty 20—40 years ago. Adiposity explained some, but not all, of these differences in timing of juvenility among migration groups so other lifestyle factors are also at play. The next Chapter explores if and how lifestyle tracks with the migration scale.

Figure 34: Median Ages of Juvenile and Pubertal Stages of Development Among Sylheti, First Generation, Second Generation and White British Girls



Each horizontal line represents a segment of the life course for each migration group which are ordered in increasing order of the migration scale. The black shapes along the x axis represents the universally accepted normal age at each stage. The coloured shapes summarises the age at each stage for each migration group.

CHAPTER 5: GROWING UP AS BRITISH-BANGLADESHI GIRLS

SUMMARY

In this Chapter I document the range of identity choices British-Bangladeshi girls make as they grow up in East London, England. In Chapter 3 I demonstrated that adrenarche occurs earliest among first generation migrants compared to other Bangladeshi and British girls, suggesting that individual migration is associated with earlier adrenarche. In Chapter 4 I demonstrated that thelarche (breast development) and pubarche (pubic hair growth) occur earlier among all girls living in the UK than Sylheti girls, suggesting that the timing of pubertal onset is associated with individual and ancestral migration. In this Chapter, I explore how migration and 'acculturation'[1] intersect with being a British-Bangladeshi girl growing up in East London. I consider in particular the following two questions: 1) does 'acculturation' increase with the migration scale and 2) to what extent does social development run parallel with stages of juvenile and pubertal development.

[1] Although widely used throughout this Chapter, the term acculturation represents a problematic concept and is thus referred to in quotations. Many studies employ acculturation variables in an effort to capture objective characteristics of a population. However, in this Chapter, I challenge the presumptions underlying acculturation and its supposed markers.

INTRODUCTION

Human adolescence is the stage in life when social and sexual maturation take place (Bogin 2001). From a social science perspective, adolescence captures the social transition from being a child to becoming an adult. Theories of childhood also suggest that the socialisation process is not necessarily linked straightforwardly with age and can occur much earlier than adolescence (James et al. 1998). From an evolutionary perspective, juvenility, marked by adrenarche, begins the increased capacity for social learning (Campbell 2006), and puberty—marked in females by thelarche, pubarche and menarche—encompasses the biological transition from a reproductively immature to mature individual. Collectively, for females these transitions are a part of growing up, a process that encompasses both being made and self-making within a particular context (Ong 1996).

Anthropologists and psychologists have looked at adrenarche and adolescence in relation to identity both across cultures and ethnicities, respectively, and two universals emerge from their work. First, adrenarche marks the emergence of the sense of self, predisposing children with the cognitive ability to engage more with adults, to learn their culture and to fulfil social roles (Lancy and Grove 2011; Nelson 1993; Campbell 2006). During adolescence, ethnic identity typically emerges as a result of an identity crisis (Erikson 1968), exploration (Phinney 1989) or encounter (Cross Jr 1995) when the sense of being different becomes salient. Not all individuals identify with their ethnicity; instead, ethnic identity is particularly salient in multicultural societies. Few studies have examined ethnic identity development in children younger than adolescents, but those that have suggest that ethnic identity emerges earlier, during the middle adolescent years (French et al. 2006) or juvenility (Bernal et al. 1990). The relationship between the development of ethnic identity and the early sense of self that emerges with adrenarche has not been previously examined.

I analyse the intersection of ethnic identity and growing up through Bourdieu's notion of habitus: "systems of durable, transposable dispositions, structured structures predisposed to function as structuring structures" (Bourdieu 1997: 72) or in other words the regulated improvisations that produce practice within and between cultural fields (Webb et al. 2002). Perhaps one of the most applicable tools of habitus to the study of acculturation in ABBY is the idea that habitus is 'of the moment' when dispositions gained from cultural trajectories meet and are constructed in practice during particular circumstances in real time (Webb et al. 2002, p.38). The observations I made of adolescent girls (as exemplified in the boxes of text throughout the chapter) can be analysed in relation to some of Bourdieu's key terms as: cultural field, cultural trajectories, bodily hexis, and doxa. The definitions of these terms, according to Webb et al. 2002, and how they apply in ABBY are outlined in Table 14.

Table 14: Bourdieu's' Terms defined and applied to ABBY

Bourdieu Term	**Definition**	**Example in ABBY**
Cultural Field	Sites of cultural practise	Girl and Woman Bangladesh and the UK Home and School
Cultural Trajectory	The movement across and between various fields that constitutes an individual's history and which therefore shapes their habitus	Migration (individual or ancestral) Growing up
Doxa	A set of core values and discourses which a field articulates as its fundamental principles and which tend to be viewed as inherently true and necessary	Gender, Religion, Ethnicity, Woman, Muslim, Bangladeshi
Bodily Hexis	The physical attitudes and dispositions which emerge in individuals as a result of the relationship between fields and habitus	Dress and Hijab Food and Diet Language and Friends

In this Chapter I will draw from ethnographic fieldwork to explore the experiences of growing up as a British-Bangladeshi girl in London. In so doing, I consider in particular the following two questions: 1) does 'acculturation' increase with the migration scale and 2) to what extent does social development run parallel with stages of juvenile and pubertal development. Experiences of identity development may be structured by gender, religion, age and ethnicity (among other things); these factors can compound when, how, and with whom one chooses to identify (Tajfel and Turner 1986; Pahl and Way 2006). For British-Bangladeshi girls growing up in East London, their bodies and selves experience change along two contrasting conditions: growing up – from child to woman – and 'acculturation' – from Bangladeshi to British. Yet, the concepts of growing up and 'acculturation' assume linearity and imply that one must leave the first state in order to enter the next. Migrant studies are often designed to test the effects of 'acculturation'. Migrant populations can be classified as existing between two cultures, assimilating into the host culture and dissimilating from the culture of origin. This implies that migrants are passive as they are 'acculturated' into the host culture and that 'acculturation' follows a simple unidirectional process (Pollard 2011). An alternative approach is to study the agency and active role migrants display in their daily lives and how this relates to the intersection of their health and migration history. The larger social structures and dynamic social process that influence behaviour should also be considered, rather than separated, from notions of culture (Hunt et al. 2004).

Using detailed ethnography and questionnaire data collected from both the UK and Bangladesh, I explore markers of 'acculturation' including language, peer friendships, food and dress among Sylhetis and British-Bangladeshis and relate such markers to the process of accepting, rejecting and creating identities while growing up. I explore the relationship of social and sexual maturation by comparing potential cultural markers of growing up among

all Bangladeshi girls using age and juvenile/pubertal stage. In describing the experience of growing up and 'acculturation' among the participants, I build on Jessica Jacobson's (1998) ethnographic work among Pakistani youth in London, which highlights the central role of religion in British-Asian lives. Another starting point for this Chapter is Katy Gardner's research on Sylhetis in London (Gardner and Shukur 1994). Both Jacobson and Gardner assert that British-South Asian youth identify with religion as opposed to other forms of identity. Primarily through a detailed ethnography of dress and food, I explore how girls accept and reject preconceived identities and create new identities, although I also review other markers of identity such as language and peer friendships. As Ulijaszek summarises in his 2007 article, few studies have examined how identity is related to dietary behaviours and obesity and this Chapter begins to look at British-Bangladeshi identity and the valuation of food. In the discussion that follows, I adopt Pierre Bourdieu's notion of habitus to explore how British-Bangladeshis create unique identities through improvisation within the boundaries of multiple contexts: ethnicity, religion and gender.

METHODS

STUDY DESIGN

As described in detail in Chapter 2, the ABBY Project used mixed methods to explore growing up and migration among a total of 500 girls: 300 living in the UK and 200 living in Bangladesh. Girls were stratified into groups according to individual/ancestral time lived in the UK. The following abbreviations are used after quotations: White British (WB), second generation British-Bangladeshi (BB2), first generation British-Bangladeshi, (BB1) and Sylheti (S).

Gardner writes, "The blanket category 'Bangladeshi' is increasingly obsolete" (1994:158). British-Bangladeshi identity and migrant strategies are constantly in flux and the ways of dealing with changing circumstances are not homogenous within the population (Gardner 1994). I employ the term British-Bangladeshi to identify particular individuals of Bangladeshi descent living in the UK. I accept that this term may be regarded as contentious, since members of this community may not in fact refer to themselves in this way or as British or Bangladeshi. However, as Jacobson (1998) similarly argues in her book about British-Pakistanis, the term British-Bangladeshi helps to inform the reader that the people whom I describe are of Bangladeshi descent and have British residency. I also use the following terms: 'migrant' to refer to first generation Bangladeshis to the UK, 'migrant groups' (or 'British-Bangladeshis') to refer to Bangladeshi migrants to the UK and their British-Bangladeshi descendants and 'migration groups' to compare Sylhetis, British-Bangladeshi migrants groups and white British girls. Later in the Chapter I contextualise two local terms for two specific groups of British-Bangladeshis: *Londoni* (the Bangla term used in Sylhet for Sylhetis living in the UK) and *Freshis* (first generation migrants from Bangladesh to the UK).

QUESTIONNAIRE

The study questionnaire was administered through an interview and selected variables related to household socio-demographics and at the individual level: migration history, 'acculturation' and diet. Markers of 'acculturation' and ethnic identity were measured in the questionnaire by asking about self-reported ethnicity, language spoken at home and preferences regarding dress and friends (Bhui et al. 2005). Many girls did not understand the terms 'ethnicity' or 'ethnic group'. The questions that asked about friendships in relation to ethnicity were subject to data collection error because of the limitations in the wording of these structured questions (Bryman 2008). Despite the limitations, I still chose to analyse these standard acculturation questions and converted some into binary variables according to

Bhui and Colleges (2005). The concept of ethnicity was explored further through the qualitative methods described below.

Specific sections of the questionnaire explored dress and food consumption in more detail. Asking about preferences in dress and friends in relation to ethnic groups has been used previously as a measure of 'acculturation' with children living in East London (Bhui et al. 2005). To assess the type of foods that children eat, a 24-hour food recall was designed to ask about food consumption over the course of one day by asking about seven specific meals and meal times including: breakfast, a mid-morning break during school time, lunch, snacks on the way home from school, at home after school, the evening meal and food eaten before bedtime. This last meal was included specifically to capture the late night meal which is typically eaten by Bangladeshi families (Lofink 2012). The dietary recall also included supplementary questions regarding typical food preferences. This food recall method captured food types, patterns and preferences of food.

PARTICIPANT OBSERVATION

The details of participant observation have been previously described in Chapter 2. The findings included in this Chapter were mainly derived from the Fit-4-Life after-school club which was conducted for 36 weeks with Year 10 girls, aged 14 – 15 years (n=20).

The Fit-4-Life club provided me with the opportunity to explore the experience of growing up by taking part in informal discussions and by facilitating focus groups. During the sessions I made mental notes and recorded jotted notes; after the sessions I recorded full field notes (Lofland 2006). By being present at the school on a regular basis, I also had informal interviews with school administrators, teachers and social workers who helped to facilitate the research. My conversations with them were also recorded in field notes.

By facilitating after-school clubs in the UK, certain themes emerged around dress and food. I organised three focus groups to explore these themes with Fit-4-Life girls (n=12). I held two additional focus groups with parents to discuss growing up (n=10). In Bangladesh, I sought comparative data around the themes of dress and food by organising three focus groups led by field assistants.

Focus Groups about Dress and Food

In the UK, I held two focus group discussions about the meanings and reasons behind wearing a hijab with Fit-4-Life girls during a club session. I asked questions including: When should a girl wear a scarf? Can you play sports wearing a scarf? Why do you wear or not wear a scarf? In Bangladesh, field assistants held two focus groups and asked participants questions about what clothes girls wear and how this changes as they get older. Field assistants also asked girls to describe how *Londoni* dress compared to Bangladeshi girls. Field assistants reported the results to me, which I later recorded in the field journal.

In-depth perceptions and attitudes towards food, in particular Bengali versus English cuisine and the associated consumptions patterns, were discussed through two focus groups in the UK. Specifically, I asked the Fit-4-Life girls what they meant when they reported "not eating rice". (See below for an explanation of the significance of this question).

I held four informational sessions for parents at the request of some of the participating schools. I used a PowerPoint presentation to describe the ABBY Project and then held a discussion about the research. These discussions included topics such as healthy eating and pubertal development; my observations were recorded as field notes after each session.

Analytical Procedures

Measures of 'acculturation' derived from the questionnaire were analysed using content analysis which seeks to quantify content in terms of predetermined categories (Bryman 2008). The responses to these questions were coded as either binary or scaled responses following predetermined categories and compared across migration groups using summary statistics displayed in Table 16. To compare cultural markers of growing up across age groups and migration groups, wearing the hijab was turned into a binary and scaled variable. Wearing the hijab was also compared across groups according to pubertal stage. Responses to open-ended questions regarding dress were analysed using open coding where concepts were identified and grouped into categories (Bryman 2008). Similarly, field notes pertaining to dress, food and growing up were analysed using grounded theory which allows for themes to emerge from the data (Glaser and Strauss 1967). By close reading and annotation of the field notes, I identified commonalities and outliers among my observations and conversations; these were then grouped into themes. The overall theme of growing up was predetermined by me and the pre-existing methods for measuring 'acculturation' somewhat shaped the themes that emerged. The participants are identified by their migration group and age in parentheses and pseudonyms are used throughout.

Results

Socio-demographic characteristics of participants are shown in Table 15. Overall, Sylheti children had more educated parents (had attended primary school or higher) and came from a higher socioeconomic class than children in migrant groups, although more than half of Sylhetis also had relatives living in the UK.

Table 15: Comparison of Socio-Demographic Characteristics of ABBY Participants According to Migration Groups.
Results are reported as percentages or mean ±SD where noted.

Variable	Migration Group (%)			
	Sylheti	First Gen.	Second Gen.	White British
Migration Status (n)	192	44	162	50
Have visited Bangladesh	100	63	95	0
Age at migration (yr. ±SD)	NA	5.5 ± 4.3	NA	NA
Time since migration (yr. ±SD)	NA	5.5 ± 3.4	NA	NA
Londoni relatives in the UK	56%	NA	NA	NA
Household				
Household size (people ±SD)	6.6 ± 2.6	5.8 ± 2.2	5.9 ± 1.7	4.2 ± 1.3
Family owns house	52	23	42	19
Family owns a car	11	50	70	57
Single mother household	17	5	18	44
Staff	35	NA	NA	NA
Employment (part and full time)				
Father (in households with both parents)	94	67	77	76
Mother	19	21	26	44
Family Education (attended primary school or higher)				
Father (in households with both parents)	77	64	52	59
Mother	76	48	56	81

Prior studies have used language, dress and friendship as measures to explore identity and 'acculturation' among youth (Bhui et al. 2005). Food has also been suggested as a marker of British-South Asian acculturation (Gilbert and Khokhar 2008). I assessed these same measures in the ABBY Project.

Table 16 provides summary statistics of 'acculturation' markers derived from the questionnaire responses. Below, I summarise the main dimensions of 'acculturation' derived from the questionnaire responses and provide ethnographic detail for two measures, dress and food, before developing the emerging themes of accepting, rejecting and creating behaviours during the process of growing up.

Table 16: Comparison of Markers of 'Acculturation' Among Sylheti, First Generation, Second Generation and White British Girls. All numbers are reported as percentages.

Variable	Sylheti (192)	1st Gen. (44)	2nd Gen. (162)	White British (50)
Frequency of Bangladeshi Dress				
Never	2	0	1	100
Every day	45	30	6	0
2-3 times a week	48	5	15	0
Occasionally	4	24	17	0
Only special occasions	0	41	62	0
Clothes Similar to Your Ethnic Group				
No	0	16	26	14
A little	2	38	47	30
Quite a lot	22	11	19	14
Mostly	76	32	9	43
Clothes Similar to Another Ethnic Group				
No	45	21	11	62
A little	37	29	45	26
Quite a lot	15	21	20	9
Mostly	3	29	24	3
Ever Wear a Hijab (all ages)	22	86	90	0
Wear a Hijab Daily (all ages)	9	47	35	0
Language with Parents and Adults				
English only		8	26	90
Bangla (or non-English Mother Tongue)		48	28	0
English and non-English Mother tongue		45	45	10
Language with Siblings				
English		58	80	89
Bangla (or mother tongue)		11	3	5
English and mother tongue		29	16	5
Friends from Same Ethnic Group				
None or some	50	34	27	32
Quite a lot or most	50	66	73	68
Friends from Different Ethnic Group				
None or some	93	87	73	62
Quite a lot or most	7	13	27	38
Eat Rice daily				
No	11	19	22	86
Once	5	40	48	14
Twice	49	36	26	0
Three times	27	2	3	0
Four or more times	8	2	1	0
Eat rice at least once per day	89	81	78	14

MARKERS OF 'ACCULTURATION'

To address my first question, if 'acculturation' increases with the migration scale, I compare language, friends, food and dress across the four migration groups. Assuming that 'acculturation' steadily increases with individual/ancestral generations lived in the UK, one would expect patterns of 'acculturation' markers to reflect rejection of behaviours associated with the country of origin in favour of acceptance of behaviours associated with the host country. For example, with increasing individual or ancestral generations lived in the UK, migrant groups would be expected to speak more English, have more white British friends, wear clothes more like white British girls and adopt British dietary patterns. Indeed, among ABBY participants, some aspects of these standard markers of 'acculturation' follow the expected pattern; other aspects of these markers appear to contradict the unidirectional model of 'acculturation'.

Photo 2: Brick lane street sign written in English and Bangla (upper left); Tower Hamlets local council office sign marked in English and Bangla (upper right); Bangladeshi Bank branch signage in Bangla and English and advertising free service for sending remittances (lower left); East London market sign in English and Bangla (lower right).

Using language as a marker of 'acculturation' among the British-Bangladeshi community is greatly influenced by the ethnic make-up of Tower Hamlets and its school districts. Bangladeshis are, by far, the ethnic majority in the specific neighbourhoods where I conducted field work. The girls were recruited from schools where, on average, 60% of students were of Bangladeshi ethnicity; in some schools up to 86% of the student body was Bangladeshi (Chapter 1, Table 3). While London itself is a cosmopolitan city, parts of East London are quite ethnically segregated. Tower Hamlets' local authority provides many services tailored for its local British-Bangladeshi community and accommodates bilingualism in the borough. The borough circulates many communication materials about available services in Bangla, and shop signs and billboards are also written in Bangla (Photo 2). Among ABBY participants the use of English has increased with migration scale, but Bangla has also been retained, rather than replaced. Second generation girls spoke English with both

their parents and siblings more than first generation girls; however, 45% of both first and second generation girls spoke Bangla and English equally when at home. Yet, very few girls could read and write Bangla. During a focus group with second generation girls, the girls stated: "We don't speak proper Bangla"; they spoke a form of a Sylheti dialect that they compared to slang. A Sylheti girl defined British-Bangladeshis by their use of language, saying "*Londoni* are different. They can't speak Bangla like *Kamun acho* (How are you). They speak Sylheti only." Thus, the use of the Bangla language is retained but the language itself is also changing in the UK.

British-Bangladeshi youth do not appear to display the same sense of pride of Bengali culture; specifically they do not express the love of the language through poems, song and dance as is celebrated in Bangladesh. In Bangladesh there are numerous national holidays that celebrate Bengali culture through ceremonies and festivals. February 21st marks Bangladeshi National Language Day and many Sylhetis explained to me the importance of the Bangla language in their identity: they are the only country to have fought for their language. I was told on numerous occasions that Bangladesh's fight for independence from Pakistan started with the fight for its people's mother tongue. When Pakistan declared Urdu the national language, Bengalis protested, insisting that they officially be able to speak, learn and communicate in Bangla. This fight led to bloodshed and many people lost their lives in the name of the Bangla language. Bangladeshis now annually bow their heads and lay flowers at the foot of the monuments, or *Shaheed Minars*, that pay tribute to the blood lost for the Bangla language, the Bengali people and the nation of Bangladesh.

While National Language Day is a sombre day, other festivals are more joyous and celebrate the Bangla language through performance. In winter, *Pitha Utsab* (Cake Festival) takes place and community centres and schools often hold festivals. This is celebrated with cakes made from rice flour with a sweet filling and various troupes perform Bangladeshi poems, songs

and dance. People of all ages attend, but youth of all ages perform the traditional Bengali songs and dance. These performances and the celebration of Bengali culture are very different from the *Melas* I attended in London.

At the Camden *Mela*, a celebration of Bengali culture, food and music in North London, there was a main stage where Bengali traditional poems, music and dance were performed and a youth stage. At the youth stage, young people performed music and dance that was more typical of Dizzie Rascal's (a local artist) East London hip hop than anything resembling traditional Bengali music. The crowd was mostly young men interspersed with a few small groups of young women. When the announcer introduced a performance featuring a local British-Bangladeshi hip hop group, he thanked the organisers for the youth stage and acknowledged that without their sponsorship, "We wouldn't be here, you wouldn't be here. You'd be over there (pointing to the main stage) listening to some Bangla ballad!" The crowd of youth chuckled in agreement. Even though many youth speak Sylheti, the mockery of Bangla music reflects their rejection of Bangladeshi culture at large. Yet, Britishness does not replace being Bangladeshi. As I will explain next, many British-Bangladeshi youth only socialise with other British-Bangladeshis.

Making Friends and Maintaining Social Relationships in Britain

First and second generation girls reported having friends of similar ethnicity, with "most" friends being from their own ethnic group and "no, or some" friends being from other ethnic groups. It is possible that the ethnicity of friends is similar by migrant status because of the ethnic homogeneity in East London. However, many girls did not understand the term 'ethnicity' and did not identify as British or Bangladeshi, and did not categorise their friends by these terms either. Specifically, Sylheti girls reported that 50% of their friends came from different ethnic groups, reflecting subtle distinctions beyond ethnicity within the Sylheti

community. In the UK, there were also other internal distinctions within the Bangladeshi community between first and second generation migrant groups.

As Gardner previously explained, although most Sylheti migrants came from families of small-scale farmers, there is considerable heterogeneity in wealth, skills and education within the migrant groups. These differences are compounded by differences in families who were able to reunite their families quickly in the UK versus those who could not avoid the costs of long term 'international commuting'. The costs of being a recent migrant are illustrated by the socioeconomic differences between first and second generation in the ABBY sample. As seen in Table 15, 23% of first generation girls came from families that did not own their houses, while 42% of second generation girls did. A social worker told me: "There are class differences between those that stay in Tower Hamlets and those that move. The educated middle class families move on, while the more traditional get married to Sylhetis and stay in the borough". Another social worker at the Secondary School 2 (SS2) explained to me that:

> Tower Hamlets, especially [this area] has lots of temporary housing for recent migrants and that more established Bangladeshi families are moving out of the borough and further east of central London. Some of the established families maintain their houses around Brick Lane and are actually using them to generate income by renting to eastern European immigrants who are now moving to the area.

These differences in socioeconomics reflect that first generation migrants may have had the initial connection with family members in the UK to migrate, but it takes time to become economically established after they arrive in the UK.

There are also social divisions between migrants and the established British-Bangladeshi community. Consider this exchange between students and their teacher after I had asked the teacher how to recruit first generation students into ABBY:

Teacher: Was anyone born in Bangladesh?
Girl: Muhammad was. (She points to a boy across the room)
Boy: I am no Freshi (The boy gets very angry)
Girl: What? I am just answering the question.
Teacher: Calm down, why are you getting all stroppy?
Boy: She called me a Freshi. I am not a Freshi.

The term *Freshi* is the young people's term for a first generation migrant; the word carries strong negative connotations and reinforces the stigma of being a recent migrant to London. The migration pattern from Sylhet to the UK has changed in the last 20 years. Most spouses enter the UK with marriage visas and start their families once they have arrived, so very few children actually migrate nowadays (Achato et al. 2011). At Secondary School 2 there was a group of "Freshi" girls who had all recently moved to East London from Bangladesh. They were good friends with each other but did not interact with other students even though the student body was 95% Bangladeshi. They spoke very little English and were far behind in their coursework as the teachers were unable to give them extra attention; this only led to further segregation of these girls. Such segregation and stigmatisation may be a source of psychosocial stress, particularly to first generation migrants. It not only initiates a stress response but may also explain first generation migrants' earlier age at adrenarche, as previously described in Chapter 3.

Other Freshis were able to integrate more with their peers, especially if they had moved to the UK very early in life. One girl (BB1, aged 14 years) told her friends that she was born in Bangladesh but moved to the UK when she was baby. Even though she was able to hide being a *Freshi*, she still told her friends not to tell anyone else that she was in fact born in Bangladesh. This illustrates the idea of passing (or being accepted): the longer a Freshi has lived in the England the more her peers will accept her and less different she feels from her peers. But rather than adopting behaviours associated with being British, she passes by associating with other British–Bangladeshis. Another girl (BB1, aged 16 years) also born in

Bangladesh, felt less embarrassed about being born there, and less different than her British peers. 'She explained: "I have British friends and I don't think I'm that different from them, except for my skin, yeah". Interestingly, this girl attended a school where Bangladeshis were not the ethnic majority, highlighting perhaps that ethnic identity is context-dependent and varies according to needs or adversity (Gardner and Shukur 1994).

The way British-Bangladeshis view *Freshis* mirrors how they generally view Sylhetis and life in Bangladesh. Katy Gardner (1994) observes that, in addition to remittances, there are social commitments and loyalties that link British-Bangladeshis with their counterparts in Sylhet. Historically, these links were cemented through owning land, yet for the younger generation of British-Bangladeshis, who are less connected with the land, the ties are changing (Gardner 1994). Among ABBY participants, 90% of all British-Bangladeshis reported visiting Bangladesh at least once. The young people I spoke to recalled their visits to Bangladesh and expressed feeling different. One girl said, "They treat us like royalty; somehow they know we are not from there". Another said, "Man, it is different going back being born here. This is 21st century culture. There, it is backwards with a cow mowing the lawn". Many participants referred to life in Bangladesh as worse than that in England. The visits to Sylhet, which help maintain the links between Sylhet and London, lead to British-Bangladeshi stereotypes of Sylhetis, but Sylhetis also do not hold those that migrate in high esteem.

Sylhetis tend to hold negative views of those they called *Londoni*, seeing them as the nouveau riche: an uneducated class that goes to London to make easy money. Historically, the Sylhetis that migrate are known to be landowners, and Gardner (1994) suggests that there was no push to migrate because the landowning class was not very poor, but rather they were pulled by new opportunity in prospering England. Gardner refers to the first migrants as sojourners with the primary aim to earn money to be invested in land and housing at home (1994). But migration patterns and migration demographics have changed, and so has Sylhet.

A Sylheti doctor (S, male, aged 57 years) told me a joke: "A *Londoni* says: 'Call me Lord. I was a landlord but now that land is gone, so I am only Lord'". One college student explained how *Londoni* are referred to as *Osi*, the word for onion cutter, referring to the *Londoni* that go to England to work at low-skilled jobs in restaurants. Another medical doctor said (S, male, aged 56 years), "*Londoni* are a burden to Bangladesh; they come back and can't work". Nowadays, *Londoni* are associated not with the land in Sylhet, but with their low-skilled jobs in the UK.

Among the Sylheti girls that were interviewed, 56% had relatives living in London. Many times people told me about their brother or sister who lived in England, but they did not refer to their relatives or themselves as *Londoni* because this was not something of which to be proud. The upper class in Bangladesh view *Londoni* as an uneducated, conservative group with bad taste. A Sylheti tour guide (male) said, "*Londoni* girls act conservative in front of relatives, but outside of that they don't—believe me". A college student (Sylheti, female, aged 19 years) said, "*Londoni* build huge houses in villages and don't even live in them. You can tell a *Londoni* home because it will be really big and in the middle of a small village". Just outside Sylhet Town, *Londoni* homes stood out above the others in size and décor. Many of the remittances are spent on building these houses. Some homes symbolised their links with life abroad by including paintings or sculptures of planes as decoration on their homes. Some argue that despite the large amount of remittances sent to Sylhet, health outcomes in this State remain some of the worse in Bangladesh (Mohsena 2012). Despite the stereotypes both Sylhetis and migrant groups hold of each other, the ties between them remain as Bangladeshi goods, British remittances and transnational marriages continue to cross their borders.

The primary source of Sylheti migration to the UK revolves around transnational arranged marriage between Bangladesh-based partners moving to the UK to be with their British-

Bangladeshi future spouse (Gardner 1995). Afsana's story exemplifies how some British-Bangladeshi girls accept maintaining ties with Bangladesh through marriage. Afsana, a senior account manager at a bank in England, took a two week holiday to return to Bangladesh for her wedding to a Sylheti man. Afsana was born in the UK and had lived in Sylhet for five years when she was young. She was currently living in a seaside town in England and the groom was due to move to England with her after the wedding. Her older sister had previously married a man from the groom's family and so this marriage reinforced the links between these two families. For other British-Bangladeshi women, marrying a Sylheti man was less acceptable. For some girls, being sent back to Bangladesh to be married was a threat. One girl (BB2, aged 17 years), who had visited Bangladesh once when she was three years old said, "I will never go again, or else I will be married off". One mother of three teenage girls, who had separated from her husband, explained, "I cannot go visit my family in Bangladesh any time soon or else my brothers will arrange for my daughters to be married". Having a difficult marriage herself with an "Asian" man she wants her daughters to have a "life of freedom". She said it will be difficult for her daughters because, "Boys will date girls in England, but in the end they marry Sylheti girls". While some attitudes towards this custom are changing, British-Bangladeshi youth continue to accept and participate in arranged transnational marriages thus maintaining the links between East London and Sylhet.

In summary, it is evident that the Sylheti language and transnational marriage continue to unify the Bangladeshi population living in East London, but certain terms such as *Freshi* and *Londoni*, both words that could be considered *Banglish* (Bangla and English) creations, highlight differences within this group. As Gardner (1994) advises against using blanket terms such as Bangladeshi, one should be equally cautious when assuming that a British-Bangladeshi individual identifies with either British or Bangladeshi components. The rejection of eating Bangladeshi food is the example to which I turn next.

Walking out of the underground and onto the street, I step into the Whitechapel market in East London. The wide pavement is lined with stalls selling produce mostly imported from Sylhet. Large green beans, bunches of coriander, roots and mangos are just some of the array of produce available. I continue walking past the stalls and pass by a fish monger selling *Hilsha*, the large river fish delicacy from Sylhet. I turn right onto the now famous Brick Lane and door after door leads tourists into the "Indian" restaurants, all of which are almost exclusively owned by Sylhetis. Around the corner from Brick Lane, Al-halal Chicken, a chicken and chips shop, breaks the monotony of Indian restaurants claiming to be the best in London. I peep inside and see a group of Bangladeshi men enjoying various combinations of fried chicken and chips.

Photo 3: Panoramic of a typical high street in East London. A chicken and chip shop (Perfect Fried Chicken) is only four doors to the left of a curry house (Spice Hut).

Eating rice is the central part of a Bangladeshi meal. In Sylhet, many Bangladeshis asked me if, and how many times, I ate rice per day. The word 'rice' encompasses the other food

preparations such as meat and/or vegetable curries, which are almost always eaten with rice[2]. The meal often starts with rice and a vegetable, followed by meat or fish and ends with dhal (lentils). The actual method of eating rice and curry entails starting with a pile of rice on the plate and spooning a curry on top or just to the side of the rice. There are various kinds of curry in communal bowls on the table, but only one curry is eaten at a time. Each time one of these curries is spooned onto a plate from a communal bowl, the eater then mixes the curry into the rice using the fingers. This coats all the rice with the flavours of the curry and then balls of the mixture are placed into the mouth. When each course is complete, more fresh rice is spooned onto the plate and the next curry is mixed and eaten. Over the course of a single meal, one person may consume three or more servings of rice. Most meals over the course of a day consist of rice and this includes a late evening meal usually eaten before going to bed.

Adopting host country food habits is a usually held to be mark of 'acculturation' (Gilbert and Khokhar 2008); however, among ABBY participants there were contradictions between what girls ate and what girls said about what they ate. While British-Bangladeshi girls eat Bangladeshi food fewer times per day than Sylheti girls, most British-Bangladeshi girls eat rice at least once a day. About 80% of British-Bangladeshi girls reported eating rice at least once during the 24-hour food recall exercise. Contrary to what they reported in the questionnaire, girls would flippantly say: "I don't eat rice" or "I never eat rice", when talking to each other during Fit-4-Life club. I asked them to explain this apparent discrepancy

[2] It is important to note that the meals I observed and ate were with middle class people, usually in their homes or at weddings and so the amount and variety of food may differ depending on what a family can afford and access.

between their 24-hour food recall and their conversations with friends. Some girls actually contradicted themselves in their explanation by saying, "I don't eat it, but I had it yesterday", or: "I haven't eaten it in a month, but I had it yesterday". For these girls, the phrase, "I don't eat rice", means I don't eat rice *often*. One girl (BB2, aged 13 years) said, "I don't eat rice. Some people eat it all the time. Three times a day. At lunch, six o'clock, before sleep, like my dad, but that's because he has eaten it all his life". Mrs Begum, a teacher explained that, "The girls say they don't eat rice because back home, they eat rice six times a day. But people have to eat a lot of rice in the village because they are busy working in the field. Even I am trying to make my family eat less rice here". Therefore, the phrase, "I don't eat rice", does not literally mean that an individual never eats rice, but rather it reflects that she eats rice less frequently than Sylhetis. The phrase is one way a British-Bangladeshi girl describes how her food choices and habits differ from those in Bangladesh. Some girls acknowledged that at times they enjoyed eating rice. Maisha said, "I get sick of rice when I eat it too much because it is nice if you have not had it in a while". Moriam (BB2, aged 13 years), who usually preferred to eat a piece of fruit before bed rather than the late-night serving of rice, said there are some times when she cannot resist eating rice because her mother offers to feed her from her own hand. She described sitting on the floor being fed from her mother: "There is something about eating from her hand that makes it tastes so nice, I don't know what it is". Most times, Moriam rejected eating rice because that is something Bangladeshis do and she preferred not to identify as Bangladeshi. A mother feeding her children from her own hand is a common practice among young children in Bangladesh (Aziz and Maloney 1985), so perhaps the rice tasted better this way because it reminds Moriam of a time when she was younger.

Instead of rice, girls preferred eating foods that could be considered more 'British' than 'Bangladeshi'. Maisha requested that her mother prepare "pasta, lasagne, prawns, salad,

garlic bread or breaded fish" instead of rice. Some mothers are less willing to prepare British food for their families. Nazma complained that her son refuses to eat her "simple food" and instead buys his own groceries or eats "bad food" like chicken and chips. Many participants reported eating chicken and chips, and going to chicken and chip outlets was a frequent way to pass time and socialise with friends after school. The preference for this fast and prepared food was as much as about taste and preference as it was about a way to spend time with friends without parents worrying about their children's whereabouts. Unlike Nazma, many parents tolerated their children eating chicken and chips. In fact, many chicken and chip shops were owned by Sylhetis that specialised in serving halal chicken to the large Muslim community in East London. Gardner (1994) has previously described how social arenas in Sylhet and London are "…woven together by an elaborate series of reciprocities. Whilst the inhabitants of *Londoni* villages remain dependent upon money sent from Britain, their kin help them to reconstruct Sylhet in a Western setting" (p. 154). Chicken and chip shops are an example of these reciprocities, but they also illustrate how the western setting is being re-constructed in Sylhet. Chicken shops such as Shahjalal Chicken, named after the patron saint of Sylhet, reflect how chicken and chips is part of the British-Bangladeshi diet, but shops named London Fried Chicken in Sylhet also reflect how the Sylheti diet has incorporated this food (Photo 4). This demonstrates that the inclusion of more British foods is occurring in both migrant group and Sylheti diets.

Photo 4: Fried chicken restaurants in Sylhet Town (left) and East London (right)

Despite the inclusion of chicken and chips into the Bangladeshi diet, there is a Bangladeshi notion, as teacher Mrs Begum explained, that "you have not eaten unless you have eaten rice". This notion was illustrated by another mother, Shopna (BB1, aged 30 years), who fed her children British or Bangladeshi food depending on the time of day. When the children returned home, she made them tuna and sweet corn sandwiches, pasta or French bread pizza for a snack and then she would serve rice for dinner. In her view, rice was more substantial for a meal than the British food that she served as snacks. While migrant groups may be eating less rice than those in Sylhet, they are including British foods in the Sylheti and British-Bangladeshi diet rather than replacing rice altogether. Thus dietary patterns between Sylhetis and migrant groups may not differ as much as an 'acculturation' model may predict. On the other hand, the frequency of eating rice in addition to consuming British foods may be a dietary factor influencing the higher BMI observed among first and second generation girls when compared to Sylhetis (Chapters 3 and 4).

On a typical high street in London, I walk past shop windows displaying trousers, tops, dresses and jumpers in the trendy cut and colour for this season. Purple is on-trend this year. I hop on the D6 bus and travel further east into Tower Hamlets and alight at an East End outdoor market. Next to the faded windows of local charity shops, the windows of *sari* shops shimmer from the colourful sequinned clothes on display. *Eid* clothes are on the mannequins and this year turquoise and pink are on-trend. I continue to walk a little further and reach stalls at open air markets selling the latest trends even cheaper than the high street chain shops. Next to these stalls are other stalls selling Bangladeshi *salvaar kameez* (a complete outfit consisting of long blouse, loose fitting trousers and a scarf) and *maxi* (house dresses). The majority of Bangladeshi women observed shopping at the market are wearing *salvaar kameez* and head scarf and some are wearing *jilbab* (long overcoats without the full veil).

Photo 5: Girls resting and walking in East London

Following the predictions of the 'acculturation' model, British-Bangladeshi girls were less likely to report dressing like their ethnic group the longer they or their family had lived in the UK (Table 16). Specifically, second generation girls reported wearing clothes more like other ethnic groups and less like their own ethnic group when compared to first generation girls. By observation, both first and second generation girls dressed differently from the way

most white British girls dressed. The most striking difference being that British-Bangladeshi girls wore a hijab. Additionally, British-Bangladeshi girls reported wearing a hijab much more than Sylheti girls; wearing a hijab increased, rather than decreased, with migrant status.

Wearing British Clothes in a Bangladeshi Style

Most British-Bangladeshi girls reported dressing more like British girls than Bangladeshi girls. Only when girls actually wore *salvaar kameez*, a *sari* or *lenga* for special occasions such as Eid and weddings would they think of themselves as dressing like a Bangladeshi girl. In secondary school, some schools had strict dress codes and provided a salvaar kameez in the school colours. At UK Secondary School 2 the dress code was rather lenient and, other than requiring students to wear black, the female students wore a variety of clothes most likely purchased from their local high street chain shop. Girls wore loose fitting trousers, cotton tops and cardigans - usually long ones that reached mid-thigh. The clothes very much resembled a salvaar kameez as the trousers fit loosely and the tops hung low covering their torso and legs. Yet, the clothes were not salvaar kameez; instead girls layered their clothes with two or three different shirts, including a long- sleeved cotton top under a black t-shirt , both covered with a long black cardigan. By layering the clothes, only bare skin on the hands and feet was exposed. British-Bangladeshi girls identified as wearing British clothes purchased from the high street, yet their choice and way of wearing the clothes resembled Bangladeshi style and followed their interpretation of Islamic teachings.

Wearing the Hijab in London

Contrary to 'acculturation' theory, girls were more likely to wear a hijab if they lived in the UK than if they lived in Sylhet. The hijab, a scarf tightly tied around the entire face and covering all hair, was most commonly worn by British-Bangladeshi girls. Like any piece of clothing, girls and women wear a hijab in a number of ways depending on personal style. Most girls and women tend to wear their scarves tightly wrapped around their head, face and

neck, secured with pins (Photo 5A). These scarves can vary from solid, plain colours such as black, brown or beige, or can be brightly coloured with sparkly thread and designs. Some younger girls wore pre-sewn scarves that cover the head, neck and shoulders that are easily put on and removed (Photo 5B). Some teenaged girls arrange their hair so that the scarf sits high upon a bun and, in this case, they usually wear a tighter fitting scarf that covers their hair under the more decorative one (Photo 5C). Some, but very few, British-Bangladeshi girls wore their scarves loosely on their heads without pins to secure it; these girls often readjusted their scarf to keep their hair covered (Photo 5D). Girls may have worn the scarf less formally at home, but my observations were limited to the public sphere.

Photo 6: A) hijab scarf secured with pins B) pre-sewn hijab C) fashion hijab D) loose *gumpta* or *orna*

Overall, 21% of Bangladeshi, 86% of first generation and 90% of second generation girls reported wearing a hijab at least some of the time (Table 16). In place of the hijab, most Sylheti girls temporarily covered their head by loosely draping cloth—either their *orna* (the shawl worn with a salvaar kameez), the loose end of their *sari* or a scarf worn with the school uniform—around their shoulders and neck and pulling it up from behind. For most of the day, they did not cover their head and the orna, sari, or school scarf was left hanging across the shoulders. The hijab is an interesting cultural marker of identity in that it does not fall

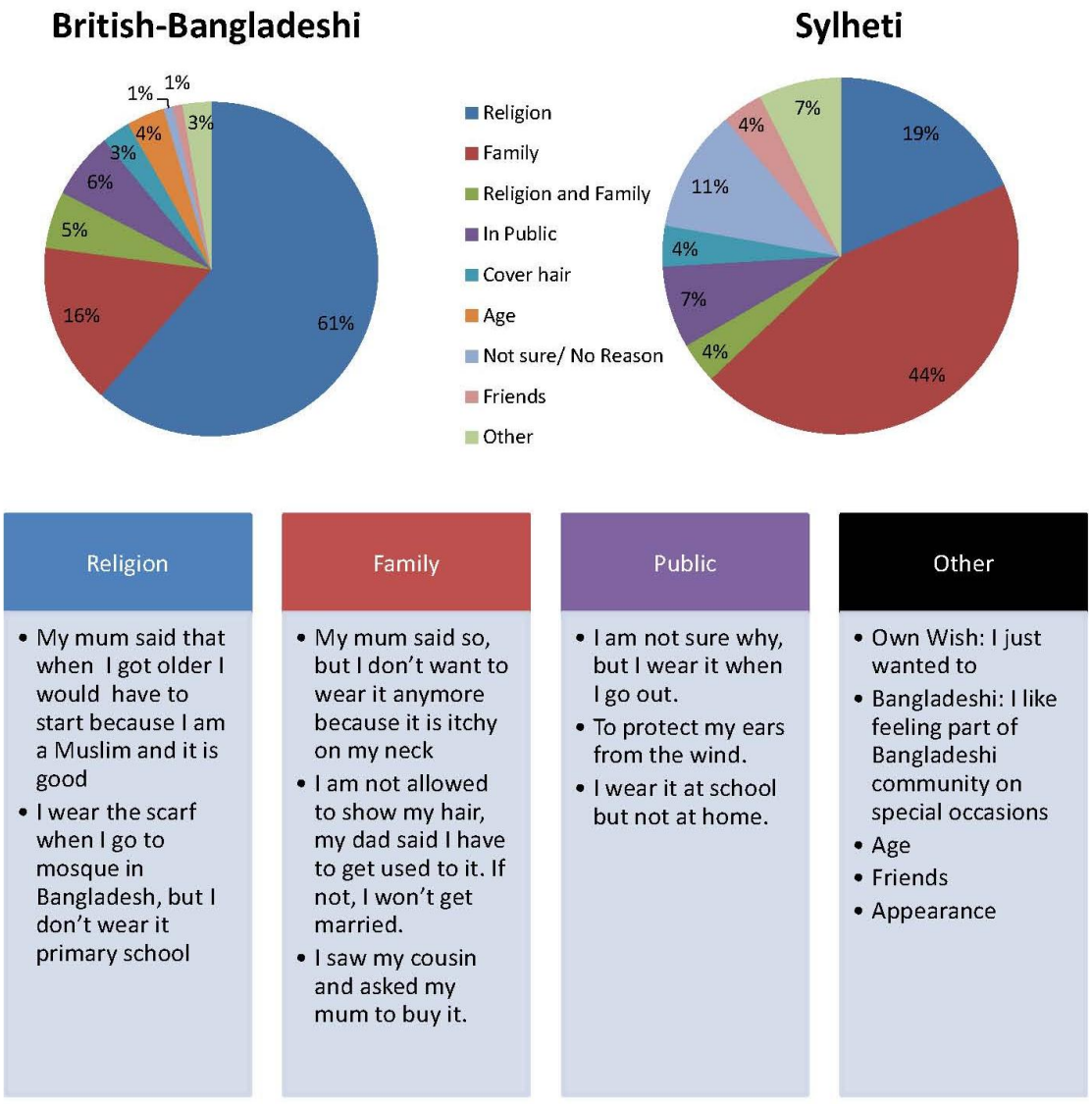
into either category of Bangladeshi or British and yet it is more likely to be worn among migrant groups with increasing generations lived in the UK. When I showed photographs of British- Bangladeshi girls to Sylhetis, most commented that all the girls were wearing hijab. One Sylheti girl (aged 10 years) specifically described the way *Londoni* dress as "shirt, pant and hijab", indicating that the hijab was a marker for a British-Bangladeshi rather than a Sylheti. Although the hijab may appear to represent 'Bangladeshiness' to a non-Muslim/non-Bangladeshi, the girls themselves explained that they wore hijab as a Muslim rather than as a Bangladeshi.

There were many reasons why girls chose to wear a hijab (Figure 35), but in the UK most girls reported wearing a hijab for religious reasons. Many British-Bangladeshi girls started wearing a hijab when they began Arabic lessons. Taking Arabic lessons in order to read the Koran is a widespread activity for Muslim-Bangladeshi children and usually entails a tutor visiting the home several times a week. Girls reported that this is when they started wearing a hijab because either their Arabic tutor or their mother told them to wear it during reading. Some girls who did not wear a hijab every day reported wearing their hijab only when reading the Koran. Other girls wore hijab regularly because they followed religious instruction to do so. One girl (BB2, age 10 years) explained she wears the hijab because "I am Muslim and it is good".

In contrast, among the 22% of Sylheti girls who wore a hijab, only 20% reported wearing it for religious reasons (the majority reported wearing it for 'family reasons', discussed below). One young woman, Taslima (Sylheti, aged 19 years), who wore a hijab, explained that one covers the head during the call to prayer "to be good when praying to Allah and to respect Allah". She also said that the Koran says to show your hair is a sin and that after learning more about Islam, she "decided to follow its guidance and cover, which includes covering my hair and wearing long sleeves".

In Bangladesh, most Muslim women and girls covered their head with a scarf when the call to prayer began, but few actually wore a hijab; instead, they covered their head, neck and chests in less formal ways. When meeting people who were their senior, young Sylheti women and girls showed respect by draping their *gumpta* over their head. Syeda (S, student, aged 22 years) distinguished between the religious hijab and respectful *gumpta*. She explained that hijab is the Arabic word for veil and, while she would cover her head with a gumpta to show respect, she refused to wear a hijab because she does not practise Islam. This demonstration of respect follows the South Asian practice of purdah. Papanek (1973) explains that purdah— the segregation of women both by physical separation from men and by the covering of the female face and body—is the most commonly used system of secluding women and enforcing female modesty in South Asia regardless of religious affiliation. Women also practised purdah by covering their heads in the presence of men who were not their kin. Taslima explained, "No man should see a woman's hair or else she will go to hell. Older women don't want to show their hair to anyone, any man. Only your face can be shown." Taslima's description reflects a specific Muslim interpretation of purdah, whereas other girls spoke how the separation from men using the hijab changes at certain stages in a woman's life. One girl (S, aged 20 years) said that women wear the gumpta over their head when they enter their in-laws house as a new wife. She will do this until she births the first child which cements the kinship relationship. Ishrat (S, age 23 years), a university student who was engaged to be married, did not wear a hijab at the time of interview but she said that she probably will once she is married because "my fiancé's family likes it and expects me to".

Figure 35: Reasons for Starting to Wear a Hijab Among Those Girls Who Reported Ever Wearing One



Parents and extended family also influence girls' decisions to wear the hijab. As opposed to the UK, where religion was the most cited reason for wearing the hijab, in Sylhet, family was the most cited reason for wearing one. In Sylhet, Taslima (S, student, aged 19 years) said, the hijab was a way for families to ensure "safety" for their daughters: "Younger girls don't know about love so they don't have to worry, but parents want older girls to wear it and be protected from most bad things. In rural areas, women have low status and so wearing the

hijab protects her and society." This notion of safety which Taslima speaks of mirrors what Papanek (1979) refers to as "symbolic shelter", which is a central feature of the purdah system in South Asia (p. 315). However, among the 16% of British-Bangladeshi girls that listed family as a reason for wearing a hijab, they did not mention safety in relation to wearing it; they followed the instruction from different family members to be obedient. While doing *mendhi*[3] at a girl's evening held at a youth club, one girl (BB2, aged 17 years) pointed to her friend wearing a hijab and said, "Hisami's good - she wants to wear her hijab. My parents aren't strict, so I don't". Another girl told me she started wearing it because her brother told her she could watch Jailbreak (a popular television show) only if she started to wear it. Another girl (BB2, aged 8 years) said, "I saw my cousin wearing it and asked my mum to buy it."

Religion and family often interact when influencing a girl to wear the hijab. In some cases, the influences of family and religion were congruent. For example, one Sylheti girl said, "If you see a girl wearing a hijab it means she comes from a religious family". Another Sylheti girl (aged 12 years) explained that while she does not wear a hijab in school, she wears one when she leaves school for religious reasons and pressure from her parents. A British-Bangladeshi girl (BB2, aged 14 years) explained that she wears a hijab to follow her mother's instructions and the Koran. Among other girls, religion and familial influences were less aligned because their families gave them a choice to wear a hijab. Taslima (S, aged 19 years) wore a hijab but had previously mentioned that her family was not religious. She later explained that, "they are religious, but not so religious minded. They didn't force me... But

[3] *Mendhi* is the ritual of applying henna, a green paste made from leaves, onto the hands and feet in decorative patterns. Once the paste dries and is removed, the skin in stained dark brown decorating the skin for 2-4 weeks.

they are happy". Maisha (BB2, aged 14 years), who also wore a hijab, said that her parents gave her the choice to wear a hijab because it is "a personal decision of when one is ready". Unlike Taslima and Maisha, Syeda (S, aged 20 years) did not wear a hijab and said that no women in her family, including her mother, aunts and sister, wore a hijab either. Syeda also made this choice. She recalled the time her mother returned from the *Hajj*, a religious pilgrimage to Mecca, and came back with hijabs for her, her sister and sister-in-law. They refused to wear the scarves and asked their mother, "Why are you wasting your money?" Thus, there is variation in wearing or not wearing the hijab in response to both religious and familial influences.

Only two girls mentioned wearing a hijab in relation to being Bangladeshi. One girl (BB2, aged 8 years) said she wore a hijab on special occasions to "to feel part of the Bangladeshi community" and another (BB2, aged 6 years) said she wears it "for mosque, because I need to learn Bangla so I can be a proper Bangladeshi girl". No one in Sylhet mentioned wearing the hijab as a part of being Bangladeshi, whereas a group of Sylheti college girls mentioned how women accentuate their eyes with a tip, a colourful dot placed between the eyes just above the brow line, as a beauty symbol and a marker of South Asian identity. However, one of these girls believed that wearing tip was sinful and contradicted wearing the hijab. These different interpretations of Bangladeshi and Muslim identity demonstrate that one's identity can differ within a population depending on which aspects of the group identity they choose to make salient.

The way Bangladeshi migrant groups dress in Britain does not follow a distinct or expected pattern towards Britishness and the hijab even appears to be an apparent contradiction of 'acculturation' theory. Style of dress—in addition to language, friends and food—can be viewed as expressions of identity rather than reduced to a misleadingly linear and unidirectional notion of 'acculturation' (Hunt 2004). Jacobson (1998) notes, in reference to

Pakistani Youth in London, that some markers of identity are more fluid than others. While some identity markers are quite malleable and provide scope for diversity through different articulations by individuals and groups, other markers of identity reflect "social structures and structures of meaning which have a real or perceived historical continuity and make up a broad and inevitably constraining context for individual expressions of identity" (Jacobson 1998, p.15). Unlike other studies where dress is a straightforward marker of 'acculturation', dress among British-Bangladeshi girls is what Jacobson refers to as fluid marker of identity that captures conscious choices of how one looks and behaves. The manner in which British-Bangladeshi girls dress seems to subscribe to the fluid type of identity marker in that trousers and tops bought in typical British stores can be worn in ways that appeal to a new urban, young and Muslim aesthetic. The hijab is also a fluid marker of identity in Britain, but it is a less flexible aspect of dress than clothes. Girls who wear the hijab reflect both structures set out by Islam and the historic relationship between South Asia and Britain. In summary, both clothes and the hijab are visual markers of fluid identity choices among British-Bangladeshi girls, the expression of which also depends on age.

MARKERS OF GROWING UP

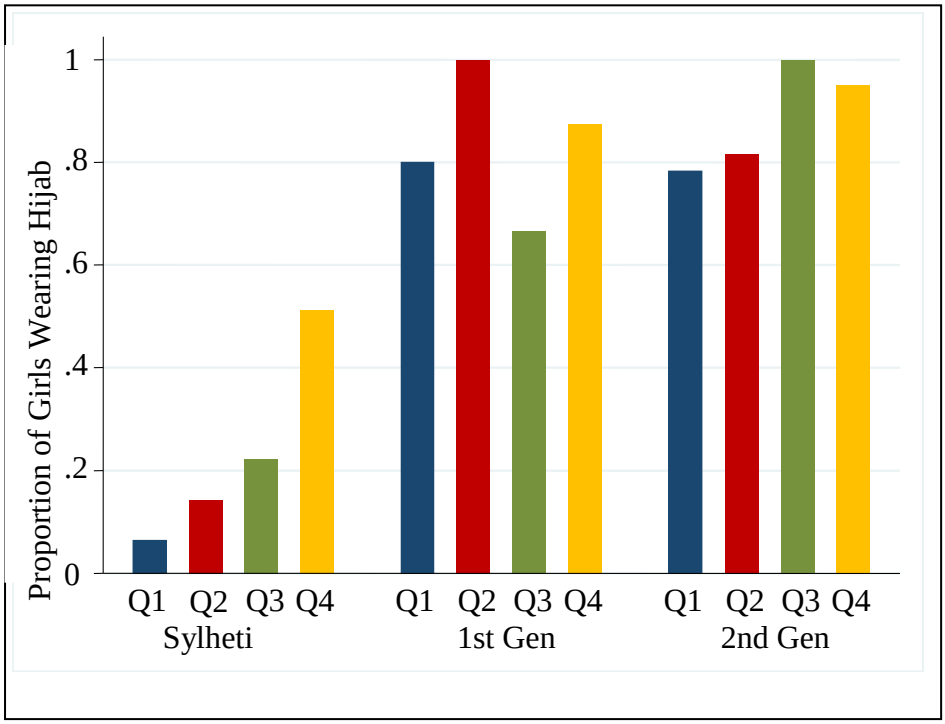
To address the second question set out in this Chapter: to what extent does social development run parallel with stages of juvenile and pubertal development, I will next explore how style of dress is also a marker of growing up and becoming a woman. There is no rite of passage or ceremony marking the transition from child to adult in Bangladeshi culture, though the practice of purdah has been linked with puberty, specifically within Muslim South Asians (Papanek 1973). I explore possible markers of juvenile and pubertal development by asking girls about aspects of their dress, specifically clothes and the hijab.

In Bangladesh, most females wear a salvaar kameez on a daily basis. The style of clothes that Sylheti females wear changes over the life course. During a secondary school focus group in Sylhet, girls explained that young girls wear "skirt, frock or pant from age five, older girls start wearing salvaar kameez around at 10 and women wear sari". The school uniforms also reflect how clothing style tracks with age. Young girls in primary school wore school dresses, secondary school girls wore a salvaar kameez and all teachers wore saris. Many mothers who waited around the school grounds to pick up their children wore a *salvaar kameez* and a couple wore *burkas*. Wearing frocks is reserved for very young girls, wearing saris is for women and the transition from wearing a frock to a salvaar kameez links with pubertal stage. A focus group of secondary school girls (Sylheti, ages 10-13 years) explained that when their breasts began to develop, they preferred to wear salvaar kameez rather than dresses so that they could drape their *orna* (scarf worn with salvaar kameez) across their chests. Because young girls wear frocks, they do not have an *orna* and do not cover their head at all during the day. There appears to be no equivalent piece of dress among British-Bangladeshi girls that marked puberty in such as clear-cut way.

Photo 7: Examples of Bangladeshi clothing including: jibab (black and grey with orange flowers) and hijab (turquoise), salvaar kameez (black and white) skirt (blue) and frock (pink).

Among all Muslim girls in Bangladesh and England, girls did not refer to a definite age or life stage that dictated when a girl should wear a hijab. Some girls as young as age five years wore the hijab, while some girls aged 16 years did not. There were differences between Sylheti and British-Bangladeshi girls in how patterns of wearing a hijab changed with age (as well as overall proportions of girls wearing a hijab). By comparing the proportion of girls that wore a hijab by age quartiles (Figure 36), it is apparent that in Sylhet, wearing a hijab increased with age in a more consistent pattern than among British-Bangladeshi migrant groups. Among Sylheti girls that wore a hijab, they were more likely to wear it the older they became. On the other hand, wearing a hijab was a less rigid marker of growing up among British-Bangladeshi girls. Many British-Bangladeshis linked wearing the hijab with particular transitions rather than getting older. For example, some girls started wearing the hijab when they moved from primary to secondary school.

Figure 36: Comparison of Proportions of Sylheti, First and Second Generation Girls Who Reported Ever Wearing Hijab according to Age Quartiles

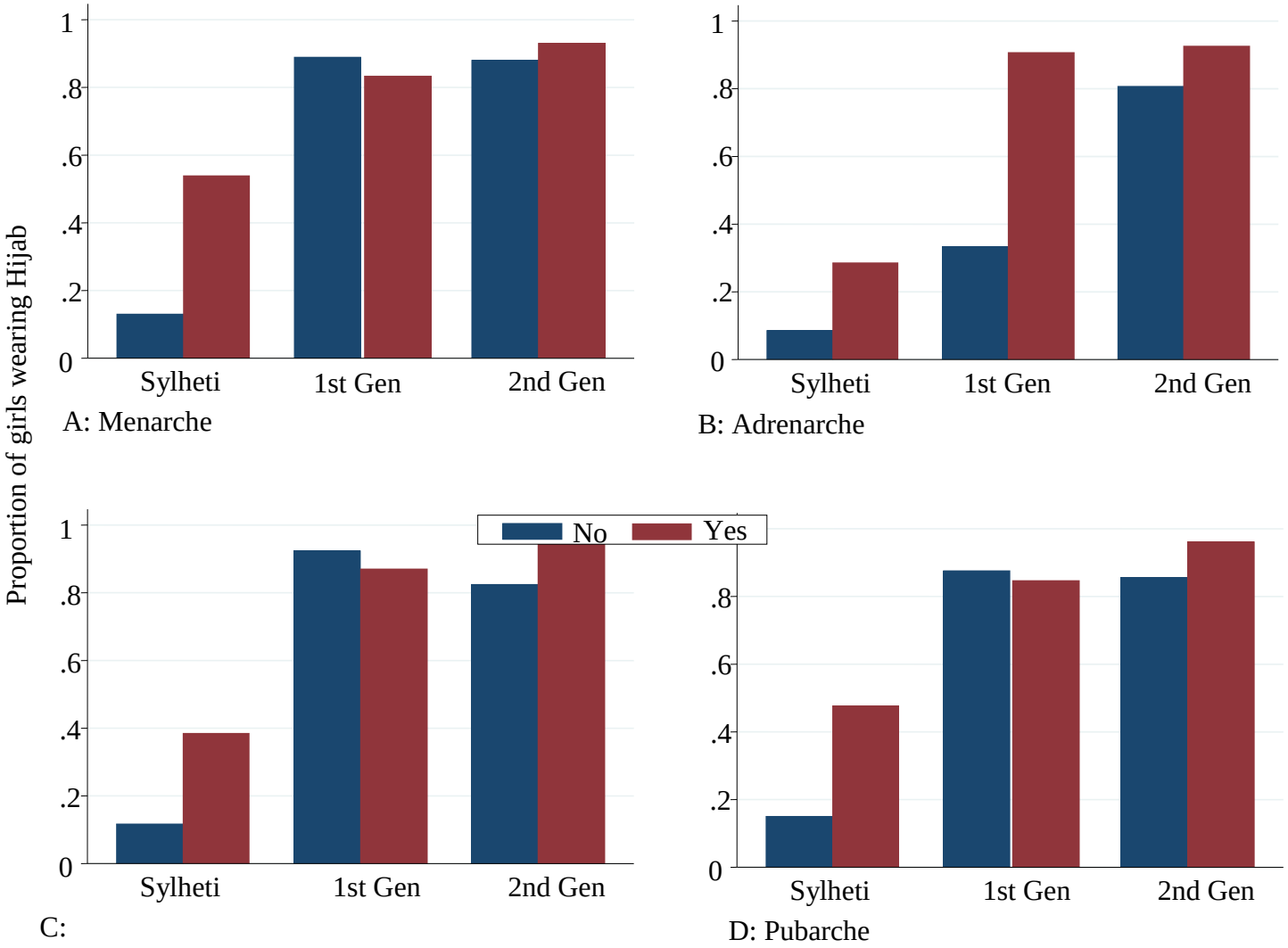


Age Quartile 1 (<7.5); Quartile 2 (7.5-<9.5); Quartile 3 (9.5-<11.8); and Quartile 4 (>11.8). The pattern increased steadily among Sylheti girls; more British-Bangladeshi girls of all ages wore hijab in the UK.

A teacher (BB2) explained that she never wore a hijab when she was in school, but chose to start wearing it every day once she went to college as there would be new people and no one would ask questions. By comparing the proportion of girls who wore the hijab between those that had reached puberty and those that had not (Figure 37), it is apparent that Sylheti girls who had reached puberty wore a hijab more often than Sylhetis girls who had not reached puberty; British-Bangladeshi girls wore a hijab whether or not they had reached puberty. However, when hijab-wearing was compared to adrenarche, all Bangladeshi girls living in Sylhet or the UK who had reached adrenarche wore a hijab more than girls who had not yet reached adrenarche. In summary, how Sylheti girls dress reflects pubertal development, but this association is not so clearly apparent among British-Bangladeshi girls. In relation to

juvenile development, wearing the hijab may reflect a process of learning culture among Bangladeshi-Muslim girls.

Figure 37: Comparison of the Proportion of Sylheti, First generation and Second Generation Girls Who Ever Wore Hijab According to Juvenile and Pubertal Status



A) The blue bar represents the proportion of pre-menarche girls who reported ever wearing a hijab and the red bar represents the proportion of girls post-menarche who reported ever wearing hijab. The other graphs show the proportions of girls wearing a hijab by those who had not but had reached adrenarche (b), thelarche (c) and pubarche (d).

Wearing a hijab varied by age among girls, but the frequency of wearing it also varied day-to-day among individuals living in Sylhet or in the UK. In school, girls wore a hijab some days and other days they did not. Some girls only wore their hijab to school but did not wear it during the weekend even if they were attending school events. I asked the Fit-4-life girls to explain the apparent inconsistent pattern of wearing a hijab. The girls explained the idea of "practising" or getting used to wearing a hijab in the process of becoming "dedicated to the scarf". Girls who did not wear a hijab everyday were practising, while girls that wore a hijab everyday were dedicated. Maisha, who wore her hijab every day, explained what being dedicated to the hijab meant:

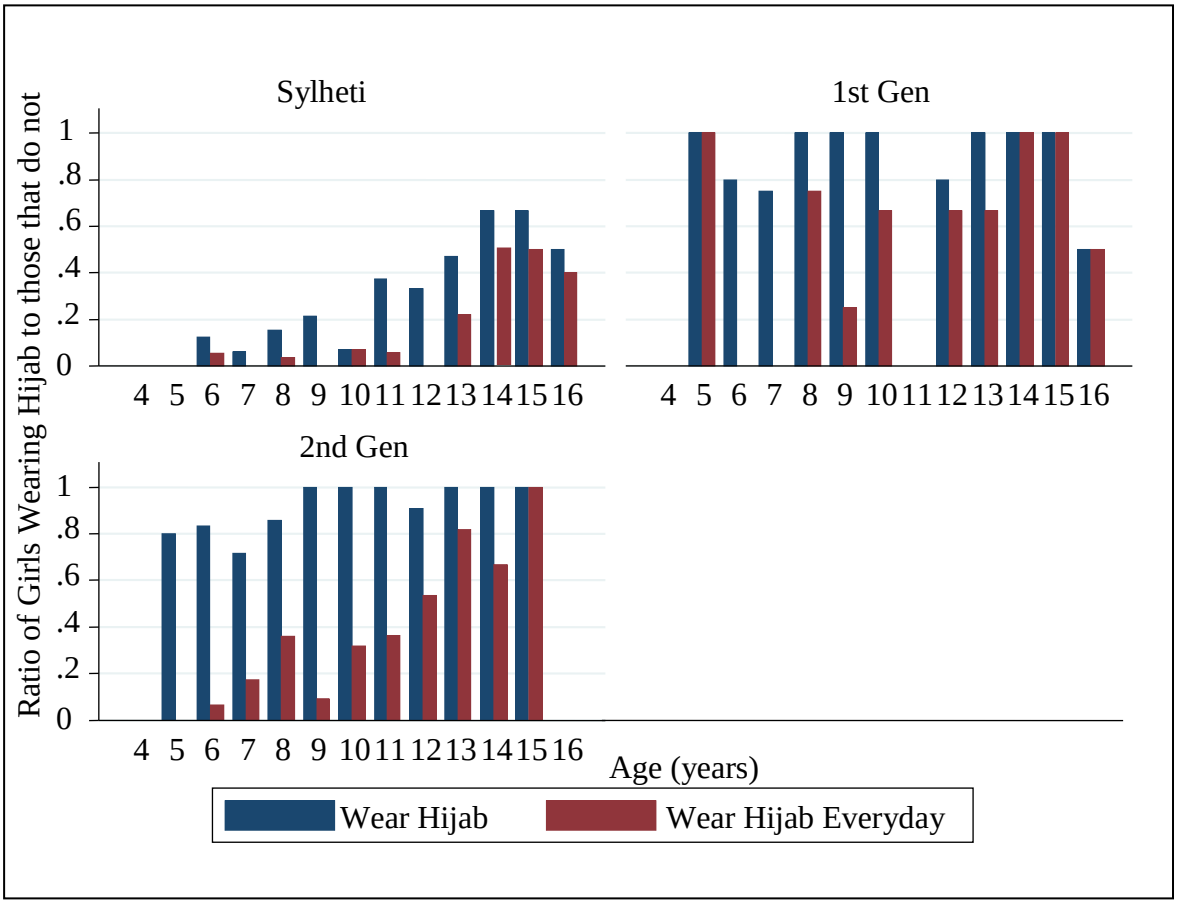
Being dedicated to the hijab means being in control of yourself, getting yourself sorted, behaving like a good Muslim... If a girl is hanging out with a group of boys and smoking weed than she shouldn't be wearing the scarf.

She further explained that "it is inside what counts", and some girls wear a hijab for fashion but this is not "being dedicated". Other girls spoke of the practising stage as "getting used to it". One girl (BB2, aged 14 years) initially started to wear her hijab in Year 6 (aged 11 years) because her mother told her she needed to practise, but she found it suffocating and so she only started wearing it properly in Year 7. A young girl spoke of playing dress up and putting on a hijab to be like a bride. Shamima (BB2, aged 11 years) said she had to get used to it for secondary school, and Fatima (BB2 generation, aged 8 years) said, "I am not allowed to show my hair. My dad said I have to get used to it. If not, I won't get married". These examples illustrate there is a period when a girl practises wearing the hijab in preparation for a later time when she will need to wear it. What they are preparing for may differ and occur at different ages, e.g. secondary school, marriage, etc., but either way, learning to wear the hijab is a process and part of growing up as a British-Bangladeshi girl.

Practising wearing the hijab can also be illustrated by Figure 38, which plots the proportion of girls wearing a hijab by one- year age categories and separately shows how many girls

report “ever wearing the hijab” (blue) as opposed to how many girls report wearing the hijab everyday (red). There is variation in the age at which one starts practising to wear a hijab as well as the age when one becomes dedicated. While there is a steady increase of “ever wearing the hijab” among Sylheti girls, there is a clear change that occurs at age 13 years when more girls reported wearing the hijab every day. However, the comparison between ever wearing and regularly wearing the hijab is different among second generation British-Bangladeshi girls: there is no pattern between “ever wear hijab” and age, but there is a gradual increase with age among girls who wear the hijab every day. As opposed to Sylhet, where some grown women wear the hijab and others do not, in East London, at some point wearing the hijab becomes ubiquitous among almost all British-Bangladeshi women. There is an underlying expectation to become ‘dedicated’ to it. The practising stages appears to occur during primary school years and parallels adrenarche and middle childhood when the capacity for social learning emerges. Some girls choose to become dedicated to the hijab when they transition to secondary school when menarche is also most likely occurring (average age at menarche is 12 years). Other girls in their final years at secondary school, such as those in the Fit-4-Life Club, are dedicated, while others say they are still practising. Fatima, who did not wear the hijab (and observably spent more time with boys than her peers), confidently said that she doesn’t wear the hijab because she is simply “not ready”. Rather than wear a hijab and contradict what it represented, she chose to not wear it. However, for Fatima and those still practising, the assumption is that one day they will all embrace the hijab in their journey to become a Muslim woman.

Figure 38: Comparison of the Proportions of Sylheti, First and Second Generation Girls Wearing the Hijab by One Year Age Categories According to Frequency



The bar graphs separately show how many girls report "ever wearing the hijab" (blue) as opposed to how many girls reported wearing the hijab "everyday" (red). Wearing the hijab every day increased around age 13 years among Sylhetis and at age 8 years and again at age 12 years among second generation girls. There was no apparent pattern in age and wearing the hijab among first generation girls, but this may be influenced by small sample size.

DISCUSSION

The apparent contradictions in the standard markers of 'acculturation' (language, friendships, food and dress) with migration scale emerge from a process of accepting or rejecting predetermined identities and creating new identities while growing up. British-Bangladeshi girls live in multiple cultures and they make choices that neither Bangladeshi nor white British girls have to make in the process of becoming women. By rejecting and accepting certain behaviours associated with either their country of origin or host country, British-Bangladeshi girls negotiate who they are and who they are becoming and, in turn, create new identities. They do this through operationalizing "habitus", the values and dispositions gained from their cultural histories that can be applied across contexts (Webb et al. 2002). Because Bourdieu tells us that these values are both durable and transposable, the girls are equipped with the tools to respond appropriately to new situations but their responses are largely regulated by where (and who) they have been in a culture (Bourdieu and Nice 1977; Webb et al. 2002). Appadurai (1997) qualifies Bourdieu's definition of habitus by suggesting that globalisation affects habitus in that it quickens the rate of improvisation. As migrant groups move through and across different cultural fields, they tend to incorporate into their habitus the values and imperatives of those fields. The constant improvisation within structures explains how markers of 'acculturation' do not capture identity among migrant groups especially during a time of identity development, such as adolescence. Thus identity is not a stable entity but is always changing, yet simple models of 'acculturation' underestimate just how dynamic this process is.

Literature on identity formation among South Asian youth living in Britain often highlights that religion remains a particularly significant source of identity. Jacobson (1998) concludes that, for Pakistani youth living in Britain, religion was more salient than the other identities available to them such as ethnicity or nationality. Similarly, Gardner suggests that a new

commitment to Islam has shifted the terms of identity from being Bangladeshi to being Muslim, but she documents examples of British-Bangladeshis identifying as British. While ABBY participants strongly identified with Islam, none specifically identified with being British. This difference between Gardner's work and my own highlights how ethnicity is not only context dependent but born from contemporary circumstances (Gardner and Shukur 1994). While Gardner (1994) acknowledges that for many British-Bangladeshis, "their experience of white racism provides a central component of their self-definition (p. 160)" and that British-Bangladeshi cultural expressions "have been powerfully moulded by their exposure to the forces of racial exclusionism", her documentation of British-Bangladeshi youth identifying as British took place before the events and aftermath of 9/11 and 7/7. Since then, the burgeoning commitment to Islam has strengthened, further separating British-Bangladeshis from mainstream white British culture, but not, as Gardner suggests, reuniting them with typical Bengali Muslim cultural roots, either (1994).

During my fieldwork, there was a threat of a racist and anti-Islamist group visiting the East London Mosque and many of the students spoke of attending the protests in defence of their community. While expressing outrage about the protest, Shamima (BB2, aged 14 years) pointed to Canary Wharf, the financial capital in London not too far off in the distance (Photo 8), and proudly said, "That is there because of us! We made that happen". Shamima was upset that anti-Islamists were coming to tell her community that they do not belong in the UK. From her perspective, she had a right to be in England as much as anyone because her ancestors had helped to develop East London. In her effort to defend against attacks on Islam, she justified her roots in England by identifying with her Bangladeshi ancestors who had lived in Britain, not Sylhet. Although the acceptance of Islam and the rejection of Bangladeshi culture is largely a result of their British experience (Gardner and Shukur 1994), British-Bangladeshi girls do not choose these aspects of their identity in efforts to become

more British. Thus, the relationship between migration, settlement and Britishness is much more complex than a simple 'acculturation' model may suggest.

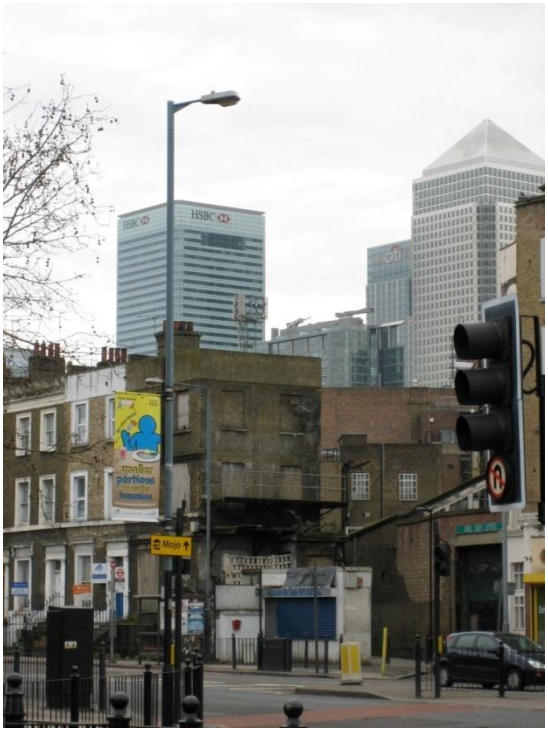


Photo 8: Street in East London with financial district, Canary Wharf, seen in the background.

Many girls held the same view as Aklima, who said, "I am proud of my religion, but not my culture". The rejection of Bangladeshi culture was evident in their denial of participating in Bangladeshi behaviours such as eating rice. Not only did British-Bangladeshi girls reject behaviours associated with Bangladesh, but they also separated themselves from people who had recently migrated from Bangladesh by marking them as *Freshis*. Still, they were proud to be Muslim. The clearest marker of their devotion to Islam was their choice to wear the hijab.

Clothes and the hijab are also markers of growing up in that they reflect changes in social maturation that generally accompany stages of development, including childhood, adolescence and adulthood. While there are no clear rules or ages when a girl changes her style of dress or starts wearing a hijab, there is some suggestion in Sylhet that wearing the

salvaar kameez and *orna* track with breast development. Among all Bangladeshi girls, practising the hijab aligns with adrenarche, the period when the capacity for learning culture increases. The process of practising and becoming dedicated to the hijab mirrors the transition that starts with the juvenile period and continues into adolescence. Even though the hijab can be viewed as a rigid marker of Islam, the practice and explanations of the hijab from British-Bangladeshi girls suggest that becoming dedicated to the hijab is a rather plastic or negotiable process that occurs during the transition from child to woman.

Even among those girls who did not wear a hijab, it was out of respect of not yet being dedicated to what it represented. By dividing ethnic identity into culture and religion, the girls were able to accept some aspects of their ethnicity and reject others so that in the end they were proud of who they were becoming. Beyond being able to claim an identity, this process of accepting, rejecting and creating their own selves was a way of being and adjusting to different circumstances in real time.

The following is a description of what took place when I organised a school field trip for the Fit-4-life girls to visit 'The Real Food Festival' in Earls Court in West London. Earls Court is located in a relatively white and wealthy part of London and, for some of the girls, it was the first time they had ventured to this part of London. This visit turned out to be yet another symbolic trip from East to West in the journey of their lives.

Entering the large convention centre filled with vendors' stalls and visitors, a group of Fit-4-Life girls screamed and giggled as they walked past the first stall of pigs in a pen. “Ewww! Gross!” they yelled, their repulsion reflecting that pork is *haraam* (or sinful) to Muslims. As the excitement wore off, and they took in their surroundings, some girls expressed feelings of being different from everyone else: "Miss, we are the only people here wearing a scarf; there are no other Asians". The act of wearing a scarf to school in and around their local borough blended in with the surroundings. Taken out of context, the scarf became a marker of difference.

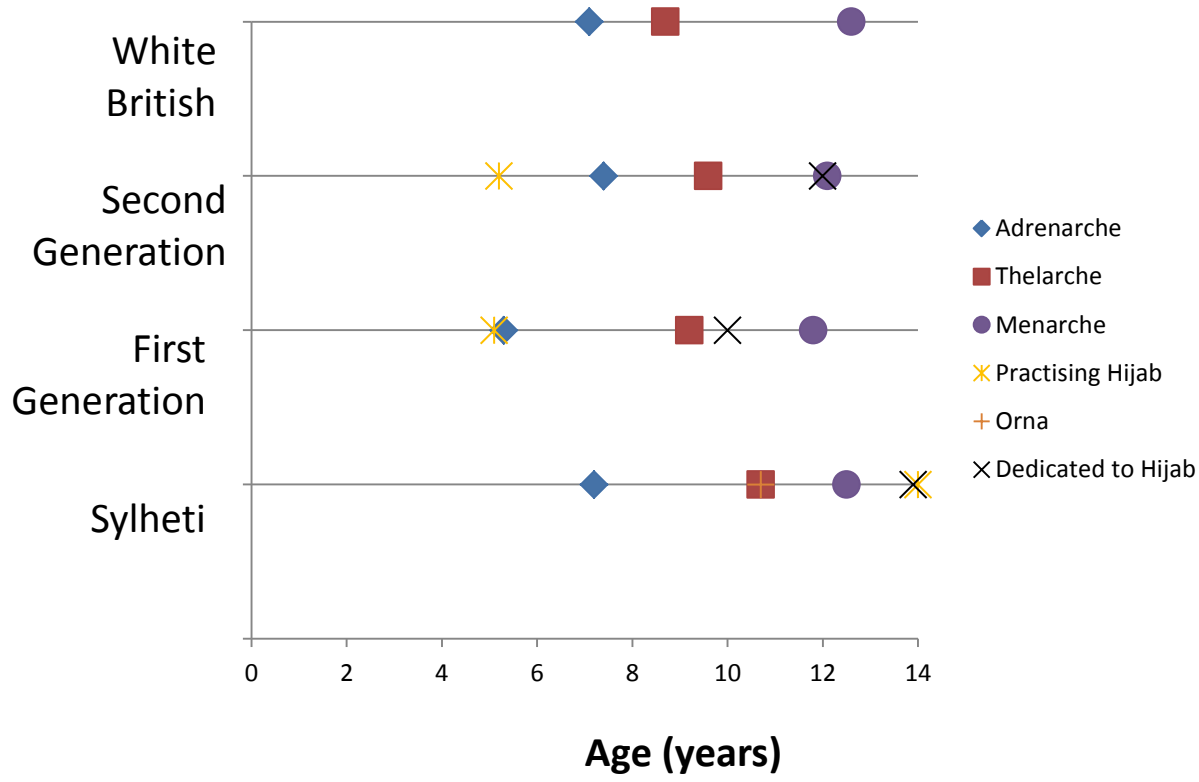
Some vendors reacted with surprise, seeing young Muslim girls at an upper-class food fair. The girls noticed others noticing them, and rather than acting shyly, they capitalised on their uniqueness by engaging more with the event. The event's organisers, spotting this group of *hijab*-clad youth, singled out the group of girls and asked them to pose for photographs and participate in a video interview. They invited them to meet an author who instantly interacted with, what he found to be, an enthusiastic, engaging group.

During a talk about food waste and sustainability, the author asked the girls what was their favourite fish to eat. The girls raised their hands with much enthusiasm and answered, "I know, I know, salmon!”. I asked them, "Don’t you like that Bangladeshi fish? What it is called?” “Oh yeah, Hilsha”, they mumbled, again dismissing a food that is a mouth-watering pride for many Sylhetis. Yet, as they continued to visit stalls and sample foods, they unfailingly inquired if the food was *halal*.

The rest of the day was spent interacting with adults behind the stalls, explaining themselves and their school with pride. As they exited Earls Court, they spotted another pig, this time a PETA protester dressed in pink fur. Rather than run away, they ran to the pig character, hugged it and posed for photographs with a sign: “Love me, Don’t eat me”. They were proud to tell others passing by who they were and what they stood for. On the tube ride home I asked girls to recap their day and what their favourite food was that they had tasted. The risotto *rice* balls were unanimously their favourite.

In some ways, this school trip was the very encounter that ethnic identity theorists (Cross 1995) refer to in that it was the first time some of these 14 year old girls faced being an ethnic minority. As the Fit-4-Life girls transitioned from being in the numerical ethnic majority to a minority in one afternoon, they negotiated and acted in a way that was something neither "Bangladeshi" nor "British" but embraced being young, unique and Muslim. In a novel setting such as a professional conference, where they were an ethnic and age minority, the girls interacted confidently with people different from themselves. They acted with confidence derived from the same pride in being Muslim that I had observed throughout the 18 months of field work. Their identity operates as habitus within the doxa (core values) of Islamic religion, Bangladeshi culture and female gender—all aspects of life which British-Bangladeshi girls pull from and press against in the process of growing up in East London.

Figure 39: Cultural and Biological Markers of Growing Up



Practising hijab (yellow asterisk) and becoming dedicated to hijab (black X) is disassociated among British-Bangladeshi girls, with practicing wearing hijab emerging with adrenarche and becoming dedicated occurring later, during or after puberty. British-Bangladeshi girls living in the UK start practising hijab (yellow asterisk) before juvenility, whereas Sylheti girls start practising hijab after puberty, however Sylheti girls begin wearing orna (orange cross) at the same time as thelarche (red square).

CHAPTER 6: CONCLUSIONS AND DISCUSSION

SUMMARY

The ABBY Project explored growing up from a biocultural perspective. In this thesis, I applied life history theory to explain differences in the timing of juvenility and puberty across three variables of ethnicity, ecology and migration status. I also questioned whether 'acculturation' increased with more individual/ancestral time in the UK and explored whether social development runs parallel with stages of pubertal development. The preceding three Chapters presented variations in the timing and experiences of various stages that constitute the transition from child to adult. This Chapter draws conclusions from the findings of the project as a whole, at both the proximate and ultimate levels of inquiry. The ABBY findings extend to the field of developmental origins of health and disease where early life trade-offs of resources predict adult health outcomes.

ABBY PROJECT SUMMARY

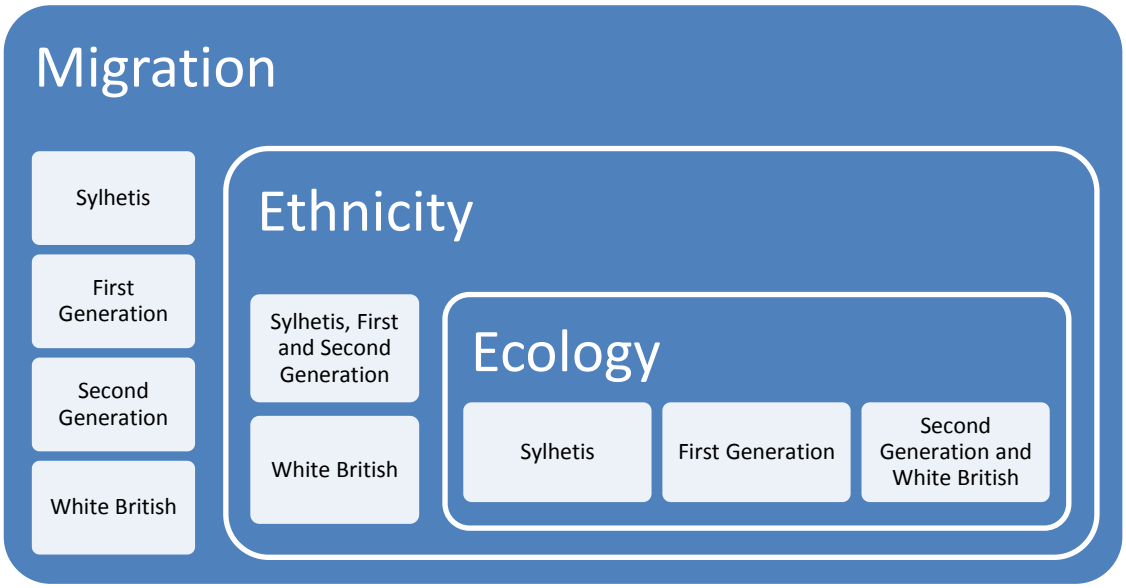
The four populations included in this study can be classified by differential exposures to socio-ecological factors depending on individual and ancestral time lived in the UK (Figure 40):

- Sylhetis were ethnic Bangladeshis, lived in a relative ecologically stressed environment and experienced no change in socio-ecological factors between parents lives and their own, and neither within their own life until enrolment.
- First generation British-Bangladeshi girls were ethnic Bangladeshis, lived in a relatively ecologically abundant environment, but experienced a change in socio-ecological factors during their early life.
- Second generation British-Bangladeshis were ethnic Bangladeshis, lived in a relatively ecologically abundant environment, and whose parents experienced a change in socio-ecological factors at enrolment.
- White British girls were ethnic Europeans, lived in a relatively ecologically abundant environment, and whose parents' experienced similar socio-ecological factors.

Differences in the timing and tempo of sexual development were compared between:

- All Bangladeshis and white British girls to test for ethnic differences,
- Sylhetis and first generation migrants as well as Bangladeshi-born and UK-born Bangladeshis to test for differences according to ecology,
- and among Sylhetis, migrant groups and white British to test for differences according to migration scale.

Figure 40: The ABBY Project Groups According to Ethnicity, Ecology and Migration



The ABBY Project examined the timing, tempo and experience of juvenility and puberty along three contrasting conditions. Comparisons according to migration (outer box) tested for differences among Sylhetis, first generation, second generation and white British girls according to increased individual/ancestral generations lived in the UK. Comparisons according to ethnicity (middle box) tested for differences between ethnic Bangladeshis and ethnic Europeans. Ecological differences (inner box) were compared in two ways: between Sylhetis and first generations and between Bangladeshi-born and British-born girls.

The findings from Chapters 3 and 4 can be summarised as future testable hypotheses: 1) a change in ecology in an individual's lifetime alters the timing of adrenarche, 2) increasing exposure to abundant socio-ecological conditions accelerates the timing of pubertal onset, 3) discontinuity in ecology, either in an individual lifetime or inter-generationally, alters menarcheal timing. These hypotheses are based on the following findings as summarised below.

The timing of sexual development differed among the four groups under study. First generation migrants reached adrenarche two years before all other girls, suggesting that the timing of adrenarche may be altered when there is a change in socio-ecological factors during an individual's lifetime (Chapter 3). All girls living in the UK reached thelarche and pubarche at younger ages than girls living in Sylhet, suggesting that pubertal onset occurs

more rapidly with increasing individual/ancestral generations lived in the UK (Chapter 4). Age at menarche was statistically similar among all girls, but descriptively appeared earlier among first and second generation girls. Contrary to the predictions of this study, migration groups did not differ in urinary oestrogen metabolite levels (Chapter 4). ABBY was able to detect statistically significant results, indicating that the study had adequate power to determine differences in aspects of juvenile and pubertal development. However, it is possible that the small samples of first generation and white British girls were subject to selection bias leading to false positive findings (in the case of age at adrenarche) or undetected differences (in the case of menarche). Therefore, the findings of ABBY need to be replicated in studies with larger, more representative samples.

Lifestyle factors, such as language, food, friendship and dress among migrant groups did not increasingly merge to match British ones (Chapter 5). Migration did not result in becoming more British, but rather girls improvised and negotiated identity options that resulted in fluid identities unique to British-Bangladeshi girls. Identifying with British-Bangladeshi girls was particularly difficult for first generation girls due to stigmatisation within the British-Bangladeshi community. This stigmatisation may be a source of psychosocial stress among the first generation migrant girls.

Cultural markers of growing up, such as style of dress, mapped onto the general stages in the life course such as child, adolescent and woman (Chapter 5). Although girls in Bangladesh explained that they changed their style of dress when breast development began, this pattern was not observed among British-Bangladeshi girls. Among all groups of Bangladeshi girls, the stage of practising to wear a hijab aligned with the emergence of adrenarche. Girls began to wear a hijab at the time of adrenarche, but they did not wear it every day. Becoming 'dedicated to the hijab' was more a gradual process. For British-Bangladeshis, among whom almost all wore a hijab, the transition from practising to becoming dedicated to the scarf

encapsulated the social process of maturing from a girl to a woman. This transition also mirrored the transition between juvenile and adolescent identities when making sense of the self extends to making sense of the self in the social world. In summary, identity development, whether across a lifetime or generations, is a dynamic process with effects on behaviours that cannot be reduced into one explanatory variable such as 'acculturation'.

PROXIMATE LEVEL EXPLANATIONS OF THE ABBY FINDINGS

At the proximate level of inquiry, the findings of ABBY support complementary theories that both energetic and psychosocial factors affect the timing of life history traits. The timing of adrenarche was mediated by energetic and psychosocial stress. When comparing the timing of adrenarche among all girls, taller and fatter Bangladeshi girls reached adrenarche earlier than shorter and thinner Bangladeshi girls, suggesting that bigger girls had more productive energy (the energy available after maintenance either through better nutrition or fewer immunological insults) to invest into growth and reproductive development. The timing of adrenarche among Bangladeshi girls seemed to be more sensitive to BMI than white British girls, although a large proportion of white British girls were overweight or obese. It may be that due to skewed data the relationship between low BMI and late adrenarche could not be detected in the white British group. Among the Bangladeshi girls, a mismatch between foetal/maternal and early childhood conditions, marked by rapid weight gain, may have resulted in earlier adrenarcheal timing. Other factors that accompanied migration may also have affected the timing of adrenarche.

Adrenarche occurred earlier only among first generation girls, despite that all UK girls were generally taller and fatter than Sylhetis. A distinguishing characteristic of first generation girls from the others is that they experienced a change in psychosocial and energetic factors in their lifetime. The term Freshi denoted being born in Bangladesh, but its negative

connotation led to stigmatisation among the British-Bangladeshi community in East London. Girls who recently moved from Bangladesh encountered language barriers in the classroom and tended to socialise solely with other recent migrants. Even in schools that were composed of 80% British-Bangladeshi students, Freshis were marginalised, not knowing the accepted British-Bangladeshi cultural code. As a result, a fair amount of psychosocial stress may have accompanied migration. Psychosocial stress may elevate cortisol levels, which activate the innermost cells of the zona reticularis that respond by undergoing morphological and functional changes (Anderson 1980). The widening of the zona reticularis would lead to production of more DHEAS (Dhom 1973). A testable hypothesis emerged from this work: if first generation girls are more stressed, then they will have increased cortisol, an earlier development of the zona reticularis, higher DHEAS levels and an earlier adrenarche.

I propose that energetic changes, as well as exposure to psychosocial stress, accompanied being a Freshi in East London and interacted to create an exaggerated response to ecological conditions. This resulted in an earlier activation of the HPA axis and, in turn, an earlier adrenarche. This finding fits with the "biological sensitivity to context" (BSC) theory which has been postulated to explain earlier pubertal maturation among girls who experience stress within their households (Belsky 2000; Boyce and Ellis 2005). In the case of migration, having elevated stress due to social positioning may lead to higher susceptibility to the biological environment in the UK. This higher susceptibility would have led to a more plastic reproductive strategy. It is also possible that there is an interaction between stress and energetic status in relation to the timing of puberty. Previous studies have postulated that at the population level food shock, economic insecurity and inequality are all sources of stress conducive to weight gain (Offer et al 2010). ABBY's findings suggest that these same stressors, particularly present among migrant groups, may also affect weight gain during childhood and sexual development during adolescence.

The timing of pubertal onset as marked by thelarche occurred earlier with increasing inter-generational exposure to ecological and social conditions in the UK. Current nutritional status (as marked by BMI and waist circumference) explained some, but not all, of the differences in the timing of thelarche among migration groups. The timing of breast development seemed to vary in response to current energetic conditions more than pubarche and menarche. Breast development seemed less sensitive to psychosocial stress than the timing of adrenarche. This result seemed counterintuitive compared with ethnographic findings which found that breast growth elicited the most social attention and behavioural response among Sylheti girls, who started to wear an orna when their breasts began to grow.

In summary, some developmental life history traits were more plastic than others. The timing of adrenarche (and maybe menarche) was altered in the group who experienced change in socio-ecological conditions, whilst these endpoints of sexual development were relatively stable in the other groups. However, breast development, marking both the end of juvenility and beginning of puberty, varied in all migration groups under study. This suggests that the endpoint of the juvenile period (thelarche) may be more plastic than the start point (adrenarche) and may be mediated by energetic factors such as increased adiposity.

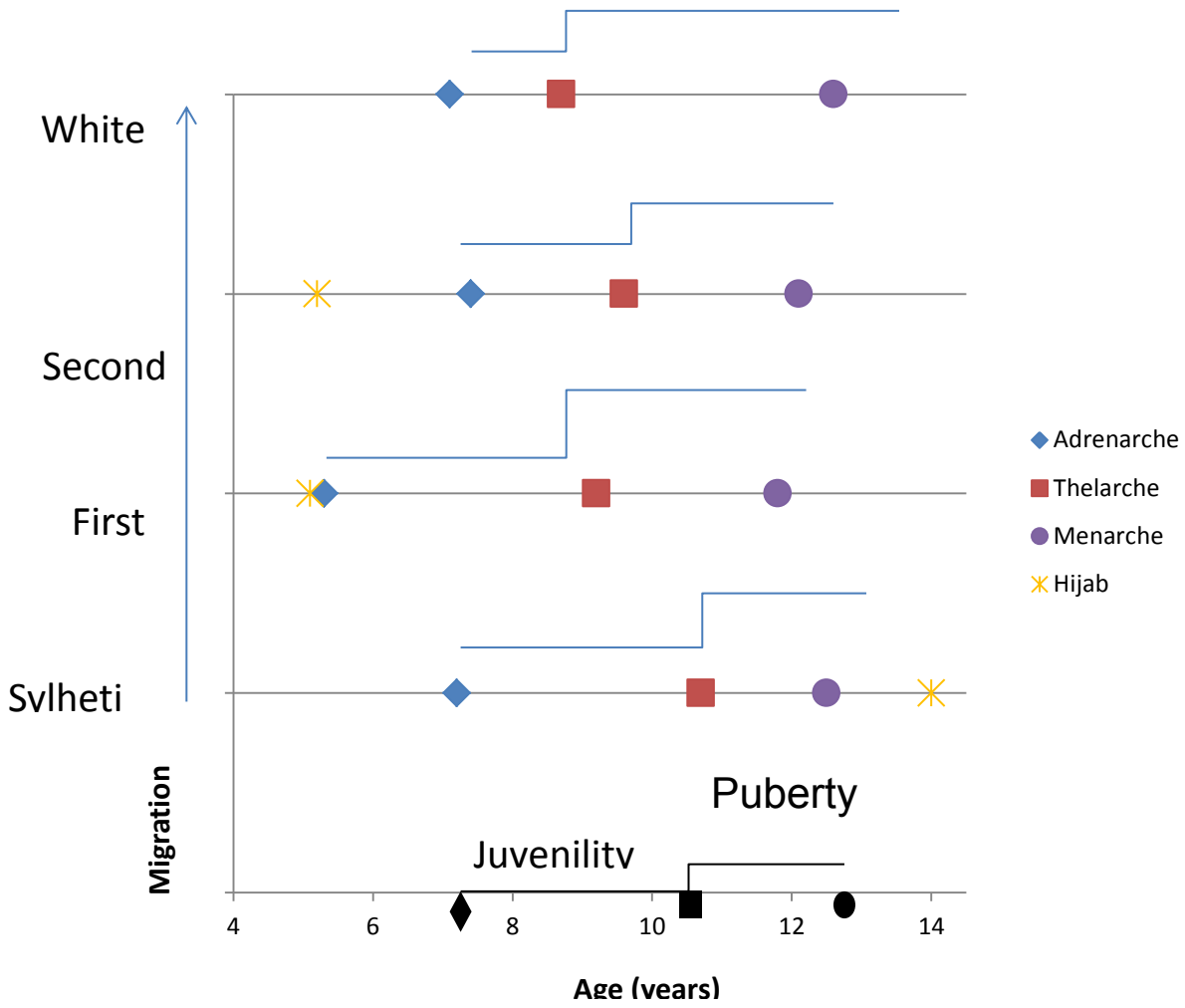
ULTIMATE LEVEL EXPLANATIONS OF THE ABBY FINDINGS

At the ultimate level of inquiry, the ABBY Project found evidence for facultative adaptation during the juvenile and pubertal periods. Life cycles differ due to facultative adaptations and ultimately evolve through natural selection during the developmental phase of life. Variation in life cycles can be categorised by fast and slow reproductive strategies; the tempo of reproductive strategy is widely assessed by the timing of puberty. The ABBY Project extended this marker of reproductive strategy to incorporate other changes: adrenarche, thelarche, pubarche, in addition to menarche, that occur early in life. By comparing the

tempo of sexual development between juvenility (the slow growth period) and puberty (the point in the life cycle when allocation of productivity switches from growth to reproduction) the lens of comparative life history can now narrow in and assess the tempo of sexual development in more detail.

Early maturation is predicted under conditions of good nutrition, low morbidity and low stress (generally defined) (Worthman 1999). In the case of the ABBY Project, which compared girls living in two countries with contrasting developmental environments, girls living in the UK should mature earlier than girls living in Bangladesh. The overall tempo of sexual development between juvenility and puberty was relatively similar among groups, except among first generation girls who had an earlier, but slower, sexual development. Sexual development appeared to be a dynamic process. When sexual development was separated into its component life stages—namely, juvenility and puberty—the tempo of sexual development differed both by interval and migration group. Among white British girls, juvenile development was fast and pubertal development was slow, whereas juvenility was slow and puberty was fast for Sylheti girls. Another way to compare reproductive strategy was by place of birth. Among girls born in the UK (both white British and second generation British-Bangladeshis), juvenile development was faster than girls born in Bangladesh (Sylhetis and first generation).

Figure 41: Markers of Juvenile and Pubertal Timing over the Early Life Course Among Sylheti, First Generation, Second Generation and White British Girls



Juvenile and pubertal tempo are depicted as step-wise stages between the stages of adrenarche (blue diamonds), thelarche (red squares) and menarche (purple circles). With the exception of the first generation, juvenile tempo is faster with increasing individual/ancestral generations lived in the UK; pubertal tempo is slower with increasing individual/ancestral generations lived in the UK. Juvenile tempo is slower among the first generation, however the pubertal tempo appears moderately paced. British-Bangladeshi girls living in the UK practised hijab (yellow asterisk) before juvenility.

Sexual development among first generation Bangladeshi migrants to the UK seemed to be the most dynamic. They developed slowly through juvenility and moderately during puberty, despite reaching juvenility and all stages of puberty earlier than Sylhetis. While first generation migrants reached menarche earlier than all other girls, the difference in age between groups was matter of months and not statistically significant. Other studies of girls

with premature adrenarche also found that menarche occurs earlier than controls, but is still within the normal range (de Ferran et al. 2011). Because the timing of menarche was relatively 'normal', earlier adrenarche resulted in a longer period of overall sexual development, meaning first generation girls had a slower overall reproductive development. Thelarche was relatively delayed, allowing for the overall tempo of sexual development to be tempered.

Humans evolved the capacity to adjust age at maturity facultatively to resolve the adaptive dilemma of variable environmental quality and to determine the optimal course of reproductive development (Worthman 1999). It is apparent that different parts of the early life cycle are more plastic, that is more sensitive to ecological factors, than others. The interval between adrenarche and menarche (sexual development) is a period of adaptive developmental plasticity for reproductive function. Specifically, adrenarche appeared to be less malleable than thelarche. My findings suggest that the period between adrenarche and menarche is a period of plasticity when early life environmental stressors can be balanced with current conditions to adjust timing of sexual maturity, especially in populations that are not nutritionally stressed. Just as juvenility may have evolved to prolong the period of plasticity in humans overall (Bogin 2001; Hochberg 2008), an earlier adrenarche may have been enacted to prolong juvenility in first generations to allow the body to account for inconsistencies in environmental conditions and to predict the best reproductive strategy with which to move forward.

Early maturation is unusual in human history, and appears to be a facultative response to a persistently high environmental quality that also predicts positive health and other functional outcomes (Worthman 1999). Yet early-maturing populations may also be at risk for certain diseases later in the life course. An earlier age at menarche is linked with increased breast cancer risk (Clavel-Chapelon 2002). The findings from ABBY suggest that earlier breast

development may explain the near six-fold differences observed in international breast cancer rates between the UK and South-eastern Asia. Premature adrenarche has also been linked with reproductive disorders such as polycystic ovary syndrome and other diseases along the metabolic pathway, such as diabetes and cardiovascular disease (Ibáñez et al. 2000). These links between early life and chronic diseases follows from studies first conducted by Barker and colleagues who found that individuals born small presented higher metabolic and cardiovascular disease later in life (Barker et al. 1989). The subsequent foetal origins hypothesis (Barker 1995) helps to inform public health efforts that identify the foetal period and infancy as the early life critical window to target metabolic syndrome health interventions. Findings from the ABBY Project suggest that reproductive health interventions should be extended well beyond infancy into middle childhood.

My study pointed to two critical periods that may affect reproductive development and adult reproductive health. Sylheti and first generation girls were both born in Bangladesh under presumably similar prenatal conditions and they both had slow juvenile development. Future studies that also compare birth weight could test whether the foetal environment affects the tempo of juvenile development. Puberty may be a critical window for adult reproductive function because, despite differences in juvenile timing across all populations, by the time of sexual maturity the differences among groups had evened out. The identification of such critical windows during middle childhood suggests that there is potential to prevent diseases, such as breast cancer and PCOS, which may have origins in early life not only in *utero* and during infancy but also during middle childhood. While in extreme cases, premature development may lead to pathology, from an evolutionary perspective, early juvenile and pubertal onset may not be harmful, but rather an adaptive way of extending the period of plasticity, allowing the body to be a better judge of its current conditions and predicting future strategies.

PUBLIC HEALTH APPLICATIONS OF THE ABBY PROJECT

The public pays almost no attention to the juvenile period, but concerns about premature puberty circulate in the academic and public arenas. The majority of discourse about puberty revolves around the worry of what earlier maturation means both socially and biologically. Earlier maturation in girls has been associated with lower self-esteem, a less favourable body image, greater rates of eating problems, depression, suicide attempts, greater association with deviant peers, norm-breaking behaviours and earlier onset of sexual intercourse (Biro et al. 2010). As Worthman (1999:152) writes, "Clinicians and the public alike are continually amazed at the psychological and behavioural precocities of contemporary youth and remain conditioned by schedules of childhood and youth that are dissonant with current patterns of maturation". Rather than highlight this period in a negative way, it is helpful to disassociate this period from age as social theorists of childhood suggest (James et al. 1998) and to view juvenility as a malleable stage, during which health promotion could have lasting impact.

The juvenile period between adrenarche and thelarche may be most suitable for health interventions, in that it is the most plastic. While anthropologists have previously demonstrated that there is cross-cultural recognition of childhood alterations when an individual becomes a juvenile, this stage in the life cycle is only now becoming more socially recognised in the public sphere. In January 2012, the New York Times published an article, "Now We Are 6: the Hormone Surge of Middle Childhood", thus bringing adrenarche into the limelight for middle class Americans. It was the most cited article that week. This article highlighted the findings of Ben Campbell and colleagues and referred to adrenarche as the beginning of "prepubescent adolescence" dislodging adrenarche from the social stigma associated with puberty. If adrenarche marks the increased capacity of the sense of self, and adolescence marks the making of that self, then the period of development between them (when children are learning and practising social behaviours) is a critical time when both

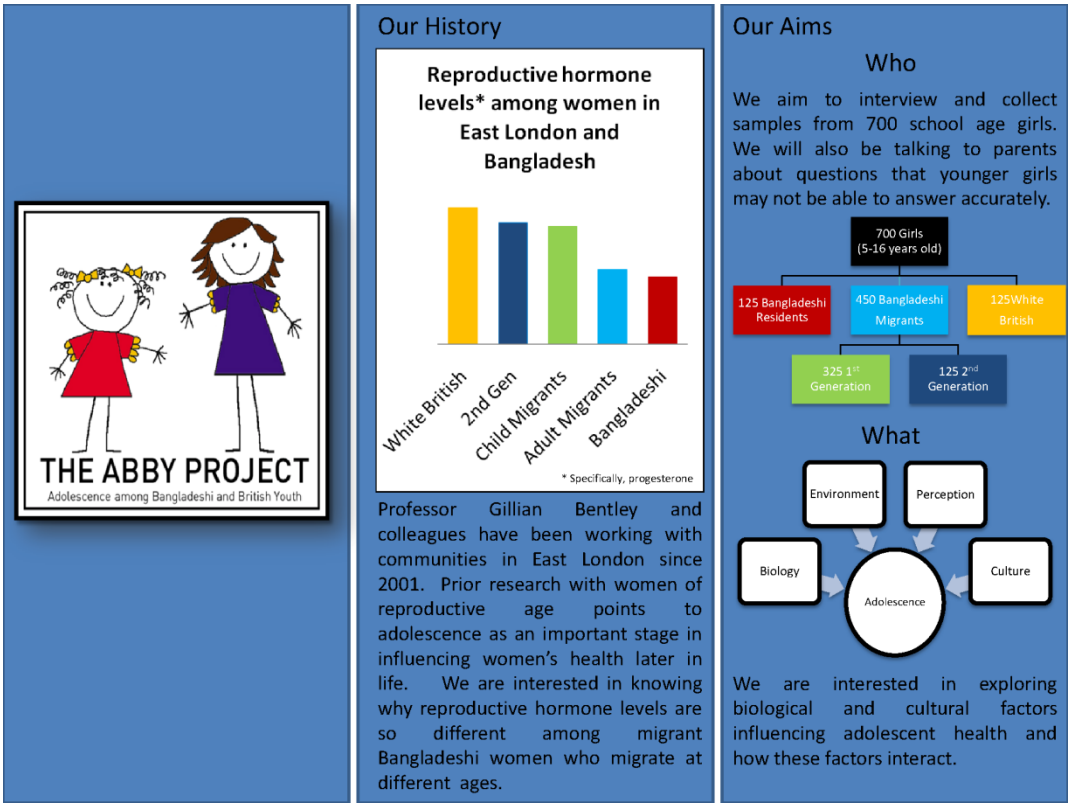
child and adult health outcomes can be improved. As evidenced by the discordance between the denial of eating rice but actually eating it and practising the hijab and becoming dedicated to it, girls are learning and experimenting with behaviours during the transition from child to adult. For example, dietary habits may have a direct impact on juvenile and pubertal timing, which may later affect adult reproductive health and disease. Collectively, the biocultural findings from ABBY contribute to the field of developmental origins of adult health and disease and contribute evidence for future studies to consider early life environments during middle childhood that may affect adult reproductive health outcomes such as breast cancer or PCOS.

ABBY'S FUTURE

Before any health interventions can be implemented, more research is necessary. The ABBY Project initially set out two objectives and my thesis addressed the first objective asking: Does juvenility differ across populations? ABBY's cross-sectional study suggests that, indeed, there is variation in the timing, tempo and experience of juvenility, yet only longitudinal studies can confirm this finding. The second objective asked: What are the determinants of juvenile timing? ABBY suggests that differences in socio-ecological factors, including energetic and psychosocial factors influence the timing of juvenility. I plan to explore further these potential explanatory variables. In future studies, I will explore the relationship between energetic factors and the timing of juvenility by teasing apart energy output (physical activity) and energy input (diet). I will also account for the perceptions of being a *Freshi* and 'eating rice' gleaned from the ethnography among British-Bangladeshi girls. Energetic status may also be mediated by differences in exposure to infectious disease in the UK and Bangladesh, therefore immunological differences such as parasite load and markers of inflammation were also collected and I will compare these markers across the

groups. I intend to explore the specific sources of psychosocial stress among first generation girls and how such stress may influence the timing of adrenarche. In an ideal world, I would continue to measure reproductive outcomes as well as energetic and psychosocial factors among the participants of the ABBY Project every five years for the next 50 years in an effort to understand more about the women they have become and will continue to become over their unique life cycles.

Appendix 1: ABBY Flyer



Appendix 2: Informational Sheets and Consent Form

Durham University

THE ABBY PROJECT

Adolescence among Bangladeshi and British Youth

Dear Parent, [redacted]

The ABBY project and Langdon Park School are working together to better understand how your daughter grows and matures. Your daughter is a healthy girl living in East London and we would like to know what makes her grow up. Her participation will help us to understand what makes other girls grow and stay healthy in England and Bangladesh.

We will be in the classroom supplementing citizenship classes. At the same time we would like to collect some information about your daughter's growth including height, weight, and hormone levels that change as she grows. This information will be part of a study among Bangladeshi and British girls in London and Sylhet. Privacy and Confidentiality will be ensured.

We hope you take advantage of this opportunity as it will provide a new learning environment for your daughter as well as helping the school and researchers better understand how girls grow.

Kind Regards,
Lauren Houghton, MSc
ABBY Project Coordinator
Durham University
07789717556

and [redacted]

If you have any further questions, please complete and return the form below:

THE ABBY PROJECT
C/O [redacted]
Name_____

☐ I would like more information regarding this project, please call me at ----------

THE ABBY PROJECT

Adolescence among Bangladeshi and British Youth

Durham University

Information for Parents

Introduction to the study: We are inviting you and your daughter to take part in a research study conducted by Lauren Houghton, Prof Gillian Bentley, Dr Mark Booth and Dr Kate Hampshire at Durham University, UK. The purpose of this study is to compare childhood development among Bangladeshi immigrants, their white London neighbours, and girls living in Sylhet, Bangladesh.

What will happen during the study: First, we will ask you questions about your age, education, health history, and current living circumstances. Then we will ask you to provide information about your daughter's birth and growth. This interview will take about 30 minutes.

Your daughter's teacher is aware of this study and has given us permission to work with children during school time. At your daughter's school we will be asking her questions about her diet, physical activity, growth and development. She may require your assistance in filling out the diet and physical activity sheets at home. In privacy we will measure her height, weight, arm width/thickness, waist and hips. We will also collect a saliva samples from your daughter to measure hormones associated with stages of pubertal development (these hormones are called DHEA-S and progesterone). We will also measure a protein in the saliva (C-reactive protein) that can measure how children respond to infections and disease. At the same time we will brush the inside of your daughter's mouth with a soft cotton brush which painlessly collects cheek cells. These cheek cells can tell us whether certain genes that control hormones during puberty are switched on or off and whether these switches change during childhood development. We will also ask girls to provide one urine sample to measure other estrogen and androgen hormones that cannot be measured in saliva. Participation in the interview and body measures will take about 45 minutes.

We are asking some girls who have started their monthly periods to allow us to collect one saliva sample every day for one month, to measure hormones that change with puberty. We will provide special collection tubes, and a separate information sheet will outline how to collect and store the samples.

How participants' privacy is protected: We will make every effort to protect you and your daughter's privacy. All researchers that will be working with your daughter have had criminal background checks and are allowed to work with children. We will not use your or your daughter's name in any of the information that we have from this study or in any of the research reports and your data will only be identified by a code number. During the study the key that tells us which code number goes with your information will be kept in a locked drawer. When the study is finished, the key will always be kept separate from the data. All our computers are also protected with passwords. Data from this study might be used for additional research later, and may be stored for several years, but will still be confidential and anonymous. Confidentiality will be maintained in all cases, except where there are any legal concerns for your child's safety, in which case the research team will have to notify the Health Visitor, GP or social services.

Risks and discomforts: There are no immediate physical or psychological risks if you or your children participate in this study, but the questionnaire may cause embarrassment because the information is personal. Being weighed and measured can also be embarrassing, but we will do our best to minimize any discomfort by providing private places in which to measure your daughter. Providing saliva samples and cheek swabs is painless and there are no known risks. Your children will receive a small gift and certificate for their time and effort in the study, and will be informed if we find any results that might indicate health problems (such as abnormalities of hormone levels).

The study will help us understand variation in salivary hormone levels during puberty, in relation to differences in lifestyle during youth. This will lead to an increased understanding of the health and physiology of girls in general and particularly of South Asian migrants.

Your rights: You should decide on your own whether or not you want you and your daughter to be in this study. If you do decide to participate in the study, neither you nor your daughter have to answer all of the questions during the interview and may stop participating in the study at any time without incurring any penalty or losses. *You will be given a copy of this Information Sheet and your signed Consent Sheet to keep prior to your participation in the study.*

Who to go to with questions: For general questions and scheduling, please call Lauren Houghton at 0778 971 7556. If you have any questions or concerns about the study please contact Professor Gillian Bentley at 0191 334 0690 .

Ethics approval: The study has been approved by the Durham Ethics Committee. If you have any concerns about your rights as a participant in this study you may contact Chair of the Durham Research Ethics Committee (catherine.panter-brick@dur.ac.uk) or send a letter to: Catherine Panter-Brick who will take complaint forward as necessary.

THE ABBY PROJECT

Adolescence among Bangladeshi and British Youth

Durham University

Information for Girls

What is the study about?

The ABBY project looks at the question of whether where you are born and and how you live affects how you develop during childhood. Because you are a healthy girl living in East London, we would like to know what makes you grow up. If you participate, you will help us to understand what makes other girls grow and stay healthy in England and Bangladesh.

What will be involved?

I will ask you questions about your life and body. In private I will measure how tall you are, how much you weigh, how big your waist and hips are and the skin on your arms. Also, I will brush a soft brush inside your cheek to collect some cells that live there. This will not hurt. Then I will give you a piece of chewing gum that will make extra saliva in your mouth. You will spit the saliva into a tube. I will also ask for a urine sample that you will provide in private when you use the toilet. Your cheek cells, saliva and urine have chemicals in them that a laboratory will measure. These chemicals will tell us about what happens inside your body. When the ABBY project is completed you will be offered a simple written summary of the results.

Is this Safe?

Nothing in this study will hurt your body. When I measure your body and ask you questions we will be in a private place. You do not have to share your measurements with anyone. I will ask you some questions about your body that may make you laugh or feel uncomfortable. If you don't want to answer them just say 'Pass'. No one will know how you answered the questions. Your name will not be connected to any of the information you give me.

Do I have to?

Your parents and teachers have given me permission to speak with you today. But you should decide for yourself if you want to be in this study. If you say yes now, but don't want to later, that is ok just tell me you changed your mind.

Questions

If you have any questions about the study you can ask me, your teacher, your parents, or you can contact Professor Gillian Bentley at 0191 334 0690 / g.r.bentley@durham.ac.uk.

Contact Information

Researcher: Lauren Houghton, Department of Anthropology, Durham University
Address: Department of Anthropology, Dawson Building, South Road, Durham DH1 3LE
Email: l.c.houghton@durham.ac.uk
Phone Number: 07789717556

Durham University

THE ABBY PROJECT

Adolescence among Bangladeshi and British Youth

What will happen?

1

I will ask you questions like:
When is your birthday?
What did you eat yesterday?

3

I will collect some samples from your mouth.

2

I will also measure your body.
Nothing in this study will hurt your body.
You will have privacy.

I will also ask questions about your body that may make you laugh or feel uncomfortable.
If you don't want to answer them just say 'Pass'.

If you say yes now, but don't want to later, that is ok just tell me you changed your mind.

Durham University

GENERAL CONSENT FORM

Please answer all the questions, and circle either 'yes' or 'no'.

Have you read the Information Sheet? YES / NO

Have you received enough information about the study? YES / NO

Do you give consent for your daughter to participate in the study? YES / NO
Do you give consent for your daughter:

to be interviewed?	YES / NO
to be measured?	YES / NO
to give a saliva sample?	YES / NO
to give a urine sample?	YES / NO

Do you understand that you are free to withdraw from the study:

* at any time and
* without having to give a reason for withdrawing YES / NO

Signed ..... **Date** .....

(NAME IN BLOCK LETTERS) .....

(YOUR CHILD'S NAME IN BLOCK LETTERS).....

Durham University

GENERAL ASSENT FORM FOR GIRLS

Please answer all the questions, and circle either 'yes' or 'no'.

Have you read or has someone explained to you the Information Sheet? YES / NO

Have you received enough information about the study? YES / NO

Do you agree to participate in the study? YES / NO

Do you agree to:
be interviewed? YES / NO
Be measured? YES / NO
give a saliva sample? YES / NO
give a urine sample? YES / NO
Do you understand that you are free to withdraw from the study:

* at any time and
* without having to give a reason for withdrawing YES / NO

Signed ..... **Date** .....

(NAME IN BLOCK LETTERS) .....

Appendix 3: Questionnaire

Contact Information	
Name	
Address: House Number and Street	
Borough	
School	
Phone	
Email	

1

2

ABBY (Girls Part) ID Code

Interviewer

_____

Date _____/_____/200__

Comments/Observations_____

The ABBY project
Questionnaire for girls

1 Personal Information

1.1 What is your date of birth?

Day Month Year

1.2 Where were you born?

Town Borough City Country
Village Union Town District

1.3 Where was your mother born?

Town Borough City Country
Village Union Town District

1.4 Where was your father born?

Town Borough City Country
Village Union Town District

1.5 Have you ever lived outside of the UK ?

- ❑ No
- ❑ Yes
  - o If yes where and at what age?

Where and Age

1.6 If you were not born in the UK, at what age did you first arrive to England?

age

1.7 Have you ever returned to Bangladesh? If so when and for how long?

age

1.8 How old were you when you moved permanently to England from Bangladesh?

age

1.9 Where did you live in Bangladesh before coming to England? If you lived in various places, please state where and your age at the time

Age	Village Union Town District

2 Socioeconomic Information

*Please answer the following questions referring to your **current** home.*

2.1 What type of housing do you live in?

- ❑ House (do you care if it's detached (single family) or not?
- ❑ flat
- ❑ other, specify

2.2 Does your family own or rent the place you live in?

- ❑ owns
- ❑ rents
  - ❑ private landlord
  - ❑ Council/ local housing authority
- ❑ I don't know

2.3 How many rooms do you have for use only by your family?

*Do **not** count bathrooms and dressing rooms or storage rooms*
Do count all other rooms, for example: kitchens, living rooms, bedrooms and studies. If two rooms have been converted into one, count them as one room.

2.4 In England, does your family own a car?

- ❑ no
- ❑ yes
  - o If yes how many?

cars

2.5 How many members are there permanently living in your current house?

number of family members

2.5.1 Please indicate their relationship to you, their ages and their occupation.

Relationship to you	Age

2.6 Does your father work outside the home?

- ❑ no
- ❑ yes

2.6.1 What is his job?

2.7 Has your father ever attended college or university?

- ❑ no
- ❑ yes

2.8 Does your mother work outside the home?

- ❑ No
- ❑ yes

2.8.1 **What is her job?**

2.9 Has your mother ever attended college or university?

- ❑ no
- ❑ yes

2.10 What is your first language or mother tongue?

2.11 What is/was your parents' first language?

2.12 What language do you usually speak at home with adults in the house?

- ❑ mainly English
- ❑ mainly mother tongue
- ❑ English and mother tongue equally
- ❑ other language, please specify _____

2.13 What language do you speak at home with your siblings?

- ❑ mainly English
- ❑ mainly mother tongue
- ❑ English and mother tongue equally
- ❑ other language, please specify

2.14 In, your mother tongue, can you:

- ❑ Read
- ❑ write
- ❑ Neither

2.15 Did you speak any English before coming to Britain?

- ❑ No
- ❑ yes

3 Physical Development Scale

The next questions are about changes that may be happening to your body. These changes normally happen to young people at different ages. Please choose only one answer for each question unless the question says otherwise.

- ❑ self-report
- ❑ interview style

3.1 During certain times, young people start to grow a lot and quickly. This is called a growth spurt. Would you say that your growth in height:

- ❑ has not yet begun to spurt ("spurt" means more growth than usual)
- ❑ has barely started
- ❑ is definitely underway
- ❑ seems completed

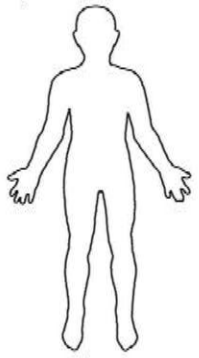
3.2 How about the growth of your body hair? ("Body hair" means hair other than on your head, for example pubic hair.) Would you say that your body hair growth:

- ❑ not yet started
  (There is no pubic hair)
- ❑ has barely started
  (There is a little long, lightly colored hair; this hair may be straight or curly.)
- ❑ is definitely underway
  (The hair is darker than before. It is coarser and more curled. It has spread out and thinly covers a larger area.)
- ❑ seems complete
  (The hair is now as dark, curly, and coarse as that of an adult female and forms a triangular pattern in the pubic area.)

3.2.1 **Has body hair started to grow on any of the following areas? (Check all that apply)**

- ❑ Underarms
- ❑ Pubic area
- ❑ Lower legs

Place an X on the figure below where your body hair is.



3.3 Have you noticed any skin changes, especially spots? Skin changes:

- ❑ have not yet started
- ❑ have barely started
- ❑ are definitely underway
- ❑ seem complete

3.4 Have you noticed your skin is becoming more oily?

- ❑ No
- ❑ yes

3.5 Have you noticed any changes in how your body smells, especially from under your arms (B.O.)?

- ❑ have not noticed any smell
- ❑ have started to smell a little
- ❑ has definitely starting to smell
- ❑ smell everyday

3.6 Have your breasts begun to grow?

- ❑ have not started
  (The breast area is flat.)
- ❑ have barely started
  (The nipple area has become larger and darker and there is a small mound under the nipple area called the breast bud.)
- ❑ definitely underway
  (The nipple area and breast are growing and are larger than before)
- ❑ seems complete
  (The breasts are fully developed and only the nipple sticks out. Breasts look like an adult's but they still may get bigger.)

3.7 Do you think your development is any earlier or later than most other girls your age?

- ❑ Much earlier
- ❑ Somewhat earlier
- ❑ About the same
- ❑ Somewhat later
- ❑ Much later

3.8 Have you begun your periods?

- ❑ If No→ **Please tell the interviewer you are finished. Fold and place this part of the questionnaire in the envelope**.

- ❑ If Yes, continue below

↓

3.8.1 How old were you when you started your periods?

I was _____ years and _____months old when I got my first period.

I was in year _____ at school.

- ❑ I don't remember exactly

3.8.2

3.8.3 Do you or have you ever taken birth control pills the hormone injection, or an implant?

- ❑ No
- ❑ Yes

If yes, when?

3.8.4 How many days are there from the first day of one of your menstrual periods to the first day of the next?

- ❑ 26-32 days
- ❑ less than 26 days
- ❑ More than 32 days
- ❑ Other _____
- ❑ I don't know

3.8.5 How long does the bleeding normally last?

- ❑ 1-2 days
- ❑ 3-5 days
- ❑ 5-7 days
- ❑ >7 days
- ❑ It changes
- ❑ I don't know

3.8.6 When was the first day of your last menstrual period? (You can refer to your own calendar or the one provided to help you remember)

Date: __/ __
DD MM

OR How many: days ago _____weeks ago _____

- ❑ I don't know

Please tell the interviewer you are finished. Fold and place this part of the questionnaire in the envelope.

4 Diet

Now I am going to ask you questions about the foods you eat. I need to know only what you actually ate this past 24 hours. You should not feel embarrassed about any food, as there are no "good" or "bad" foods. No one eats just the right foods all the time.

.

Let's start with what you ate most recently (MARK IT NUMBER 1) and work backwards until this time yesterday.

When was your last meal? Okay let's start there, what did you eat and drink at that time?

Breakfast	Break time	Lunch	On the way home from school	When you get home from school until the evening meal	The evening meal	After the evening meal but before bed	***Yesterday's meal at this time***

4.1 Did you eat or drink anything else in the last day?

- ❑ No
- ❑ Yes (please add to list and star)

4.2 Would you say this was a typical day in terms of eating and drinking???

- ❑ No
- ❑ Yes
  - If no, what was different
    - ○
    - ○
    - ○

4.3 Is there anything you usually (on most days) eat or drink that you didn't eat yesterday?

- ❑ No
- ❑ Yes
  - If yes, please list

4.4 Did you eat or drink something yesterday that you usually don't eat?

- ❑ No
- ❑ Yes
  - o If yes, please list:

4.5 What are your favourite foods and drinks and how often do you eat them?

Food	Everyday	2-3 times a week	Weekly	Once every 2 weeks	Monthly	Occasionally

4.6 Now I am going to ask you questions specifically about school lunches?

4.6.1 Are you eligible for free school meals?

- ❑ No
- ❑ yes

4.6.2 How often do you have school meals?

- ❑ Everyday
- ❑ 2-3 times a week
- ❑ 4 times a week
- ❑ Once a week
- ❑ Occasionally
- ❑ Never (skip to 4.8)

4.7 Do you bring your lunch to school?

- ❑ No
- ❑ Yes
  - o If yes what?

4.8 Do you bring snacks to school?

- ❑ No
- ❑ Yes
  - o If yes What?

5 Physical Activity

Please complete this questionnaire for the past 7 days from today until last (Insert day of week which comes after today).

What activities do you do after school?

What do you on the weekends?

How do you get to school?

What do you do in your down time?

6 General Health

We all get ill from time to time and these questions are going to ask you about times that you were not feeling well.

6.1 Have you noticed changes in your health in the last year?

- ❑No
- ❑yes

6.1.1 If the answer is yes, what has changed?

6.2 Have you been ill in the last month?

- ❑ No
- ❑ Yes
  - o If yes, what were your symptoms?

6.3 Have you been ill in the last 2 weeks?

- ❑ No
- ❑ Yes
  - o If yes, what were your symptoms?

6.4 Thinking back over the past two weeks, have you ever been bothered by any of the following? Please indicate the extent to which you are bothered over the past two weeks by any of these symptoms. [Check any that apply and feel free to add comments on the table]

Not at all =0 A little = 1 Quite a bit=2 Extremely=3

Code	SYMPTOMS	Not at all	A little	Quite a bit	Extremely	Comments
	Running/ stuffy nose					
	Stomachache					
	Diarrhea					
	Constipation					
	Headaches					
	Loss of appetite					
	Persistent cough					
	Sore throat					
	Trouble sleeping					
	Vomiting					

6.5 Did you take any medicine in the last 2 weeks?

Please list them and the reason you took them

Medicine	Reason

6.6 Have you ever had terrible diarrhea or been diagnosed with worms?

- ❑ No
- ❑ Yes
- ❑ I don't know
  - o If yes, when, where, why?

6.7 Have you ever been given medicine for parasites?

- ❑ No
- ❑ Yes
- ❑ I don't know

6.7.1 If yes,

What was the medicine?	
When did you take this medicine?	

6.8 Do you smoke cigarettes?

- ❑ No
- ❑ Yes

6.8.1 How many cigarettes/ how often?

6.9 Do you smoke shisha?

- ❑ No
- ❑ Yes
  - o If yes, how often?

6.10 Do you chew betel nut?

- ❑ No
- ❑ Yes
  - o If yes, how often?

7 Relationships

Cultural Identity Questions

The following questions are about how similar or different you feel from people in your ethnic group. What ethnic group do you belong to?

- ❑ White British
- ❑ British Bangladeshi
- ❑ Other, please specify_____

7.1 Is your choice in clothes similar to people from your ethnic group?

- ❑ No
- ❑ A little like them
- ❑ Quite a lot like them
- ❑ Mostly like them

7.2 Is your choice in clothes similar to people from other ethnic groups?

- ❑ No
- ❑ A little like them
- ❑ Quite a lot like them
- ❑ Mostly like them

7.3 If you are Bangladeshi,

7.3.1 Do you wear a hijab?

- ❑ No
- ❑ Yes
  - o If yes, how often?
    - ❑ Everyday
    - ❑ 2-3 times a week
    - ❑ Occasionally
    - ❑ Only on special occasions

When and why did you start wearing it?

7.3.2 Do you wear salvar camis or other Bangladeshi dresses?

- ❑ No
- ❑ Yes
  - o If yes how often?
    - ❑ Everyday
    - ❑ 2-3 times a week
    - ❑ Occasionally
    - ❑ Only on special occasions

7.4 Do you have many good friends who belong to your ethnic group?

- ❑ None
- ❑ Some
- ❑ Quite a lot
- ❑ Most or all of them belong to my own ethnic group

7.5 Do you have many good friends who belong to other ethnic groups?

- ❑ None
- ❑ Some
- ❑ Quite a lot
- ❑ Most or all of them belong to other ethnic groups

7.6 What I am like with my mother figure

7.6.1 Do you live with your biological mother?

- ❑ No
- ❑ Yes
  - o If no, who is your primary caretaker?

Primary caretaker

7.6.2 Do you live with your biological father?

- ❑ No
- ❑ Yes

7.6.3 At any time did a parent or caretaker live outside your home?

- ❑ No
- ❑ Yes
- ❑ If yes, who and when?

7.6.4 This questionnaire asks about what you are like with your mother – like how you act and feel around her.

7.6.4.1 One day you come home from school and your mother is not home, you are waiting for her to return. Your mother is very late.

Some kids would stay calm until their mother got there		**BUT**	Other kids would be very upset and worried about her.	
Very true for me	Sort of true for me		Sort of true for me	Very true for me

7.6.4.2 You and your mother go to a fun fair one evening. Some of the rides look a little scary, but they look fun and exciting too. You want your mother to go on some of the rides with you, but your mother says she is tired and just wants to sit on the bench and watch.

Some kids would go on the rides alone		**BUT**	Other kids wouldn't go on the rides alone.	
Very true for me	Sort of true for me		Sort of true for me	Very true for me

7.6.4.3 You are at a relative's wedding with your mother and you have to go out to the bathroom. When you come back in, there are many people around and you cannot find your mother.

Some kids would calmly look for their mother and not be too worried		**BUT**	Other kids would look for their mother and be very upset until they found her.	
Very true for me	Sort of true for me		Sort of true for me	Very true for me

7.6.4.4 You and your mother are visiting a new shopping centree to see what it is like. Your mother suggests that the two of you explore the centree together.

Some kids would only want to explore it on their own		**BUT**	Other kids wouldn't mind exploring it with their mother.	
Very true for me	Sort of true for me		Sort of true for me	Very true for me

7.6.4.5 Let's say that you have a favourite pet, a cat or a dog, that suddenly gets very ill. You are sad about it.

Some kids would let their mother know they were feeling sad		**BUT**	Other kids would not let their mother know they were feeling sad.	
Very true for me	Sort of true for me		Sort of true for me	Very true for me

7.6.4.6 One day you have a problem with a friend at school. When you get home, your mother can tell that you are upset and starts talking to you about it.

Some kids would feel comfortable talking to their mother about their feelings and problems		**BUT**	Other kids would just want their mother to leave them alone.	
Very true	Sort of		Sort of	Very true

for me true for me true for me for me

8 Growing Up

This next section, I want you to define certain terms in your own words.

How would you define Growing up?
Use your own words to define woman.
Use your own words to define girl.
Are you a girl or a woman? Explain.
What are your dreams and aspirations?

What causes you stress?

9 Anthropometrics

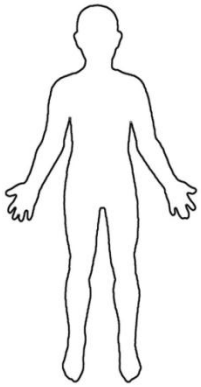
Anthropometric measure	Record Measurement below
A. Height (cm)	
B. Weight (kg)	
C. BMI (calculate as height (m) divided by weight (kg) squared)	
C. Tricep skin fold measurement(cm)	
D. Waist measurement(cm)	
E. Hip measurement(cm)	
G. Waist to Hip ratio (calculate as waist (cm) divided by hip (cm))	
F. Arm circumference(cm)	
G. Sitting Height	

Appendix 4: Adrenarche Development Scale

Classification into one of four adrenarche categories (pre, beginning, mid, advanced) were based on 4 indices thought to be salient for marking adrenarche, namely axillary hair growth and development of the sebaceous glands. The points for each response are indicated in the closed parentheses.

Has body hair started to grow on any of the following areas? (Check all that apply)
No (1)
Lower legs (2)
Underarms (3)
Pubic area (4)

Place an X on the figure below where your body hair is.



Have you noticed any skin changes, especially spots or pimples? Skin changes:
have not yet started (1)
have barely started (2)
are definitely underway (3)
seem complete (4)

Have you noticed your skin is becoming more oily?
No (1)
Yes (4)

Have you noticed any changes in how your body smells, especially from under your arms (B.O.)?
have not noticed any smell (1)
have started to smell a little (2)
has definitely starting to smell (3)
smell everyday (4)

Appendix 5: After School Clubs' Activities

GAL CLUB Activities

Week 1: Introductions and make your own Beauty Products

- We started with an ice-breaker where each girl told 2 truths and 1 lie about themselves and the group had to guess which one was the lie.
- Student ambassadors welcomed the students and told them about life at secondary school and answered any questions.
- Entry surveys were distributed and filled out to collect initial data regarding the club
- We broke up into groups and used ingredients usually found at home to create beauty products including beetroot rouge, honey-citrus face mask, baking soda exfoliator, cucumber toner, and coco lip gloss.

Week 2: Self-Esteem with Life-Coaches

- Pair share, introduce your partner: name, something interesting, where name comes from
- Picture of self from magazines: girls selected from an array of images that reflected how they feel when they are happy and sad. The girls discussed why they chose such pictures in small groups.

Week 3: Bullying with Life-Coaches

- Agree, Disagree, in the Middle: a statement was read aloud and the girls were asked to stand on a particular side the room if they agreed with the statement, the other side of the room if they disagreed or in the middle if they both agreed and disagreed. The statements covered different feelings and thoughts surrounding the experience of being bullied or bullying others.
- The generator wheel: girls explored the difference between facts and beliefs and put these into the context of "IF" statements such as If I see someone being bullied, I would have to report this or else that person would get hurt. The girls explored the different scenarios and picked apart the possible reactions and feelings associated with different bullying situations.

Week 4: Photo shoot with Carmel King Photography

- Being a photographer: Carmel explained what it means to be a freelance photographer and how she uses light to take photos
- Exploring photos: Girls browsed through magazines and chose the photos they liked and explained why
- Photo shoot: while outside the girls had portrait and group shots taken with a background of their choosing

Week 5: Healthy Cooking with Chef Greg Silverman, London Borough of Richmond Upon Thames

- Making Mayonnaise: Chef Greg, with the help of some students, turned eggs and lots of oil into mayonnaise demonstrating the amount of fat needed to make it turn thick (or the high quantity of fat in mayonnaise.
- Cooking: The girls broke into small groups and followed low-fat recipes to make salsa, guacamole, fruit smoothies, and hummus.
- Eating: The group ate together discussing what they made.

Week 6: Student Ambassadors and Celebrity Game

- Student Ambassadors returned to answer questions regarding life at secondary school

- Photos from Carmel King were distributed
- Exit surveys distributed and completed.
- Celebrity game: names of famous people were written on pieces of paper and stuck to people's forehead. Each person asked yes or no questions about the name on their head trying to guess who they were

Fit-4-Life Club Activities

- Two Truths and One Lie: During the first session we went around the room telling 3 facts about ourselves and the group had to guess which fact was false. This first meeting was a 'getting to know you session' where I asked the girls what they expected from the club and what they would also like to do during our times together.
- Lecture of the menstrual cycle: I presented a PowerPoint presentation that included information regarding the endocrine system, the hypothalamic-pituitary-ovarian axis, and hormonal regulation of the menstrual cycle. I had access to the Key Stage 4 science curriculum and designed the lecture to emphasize concepts that the girls were learning in biology class.
- Building models of the endocrine system: Girls were asked to bring in supplies from home and work in groups to create life-size models of the endocrine system.
- Menstrual cycle symptom diaries: Girls were given a menstrual symptom diary and explained how to track different symptoms associated with their cycles.
- Guest speaker on women's health: Professor Gillian Bentley visited the school and spoke in response to the girls' question: 'What hormone makes us fall in love?' This was a 'girl only' event that included a question and answer session.
- Healthy food cooking lessons with a professional chef: A professional chef cooked with the girls healthy affordable recipes, while discussing the ideas of healthy eating and their own eating preferences.
- Field Trip: The London Real Food Festival offered free tickets for 12 girls to attend this full-day event at Earl's Court. Girls navigated the food show in three groups and were given a hand-out to guide their visit.
- Fit-4-Life and Food: we discussed the differences between Real Food and Fried Chicken
- Summer Fun Day: Fit-4-Life club had a stall at the community summer festival where girls served frozen fruit desserts to community members.
- Beauty and science: Do beauty products work and if so, why? The girls broke into groups and followed recipes to make beauty products from ingredients found around the house.
- Sharing cultures: Nacirema: An etic description of an American culture was read to the girls without revealing who the Nacirema (American spelt backwards) were. The girls then wrote their own excerpts of ethnography on either British or Bangladeshi culture.

Appendix 6: Validation of Weibull Regression Survival Model

Median ages at adrenarche, thelarche, pubarche and menarche were estimated using Weibull regression models for parametric survival analysis in STATA using software described by Royston (2001). Because this data has both right- and left-censoring, a non-parametric survival estimate akin to the Kaplan-Meier estimator is computationally difficult to estimate, tends to have high variances, and there is no software for Cox-type regression modelling. In contrast, the Weibull model easily handles right and left censored data and allows for regression modelling. Furthermore, the Weibull model assumes an underlying shape for the age at onset distribution, so it can more efficiently estimate the median ages at onset.

We assessed the goodness-of-fit of the Weibull distribution graphically (Figures A-D). The figures plot the Weibull survival curve for age at adrenarche, thelarche, pubarche and menarche (shown as the solid line in each Figure) for each migration group against the survival curve based on a non-parametric estimate akin to the Kaplan-Meier estimate (shown as crosses in each graph). In particular, the x-axis is log (age) and the y-axis is cumulative incidence plotted on the complementary log-log scale of the survival. On these plots, the Weibull model survival curve is always a line (solid) and the dotted lines are 95% point wise confidence intervals. The estimates from the non-parametric survival curve are plotted as individual crosses (+). The non-parametric estimates (crosses) fall very close to the Weibull line for almost all ages. Thus, the Weibull model fits well for each stage of sexual development (adrenarche, thelarche, pubarche and menarche).

Figure A: Comparison of Weibull and Non-Parametric Models of Adrenarche

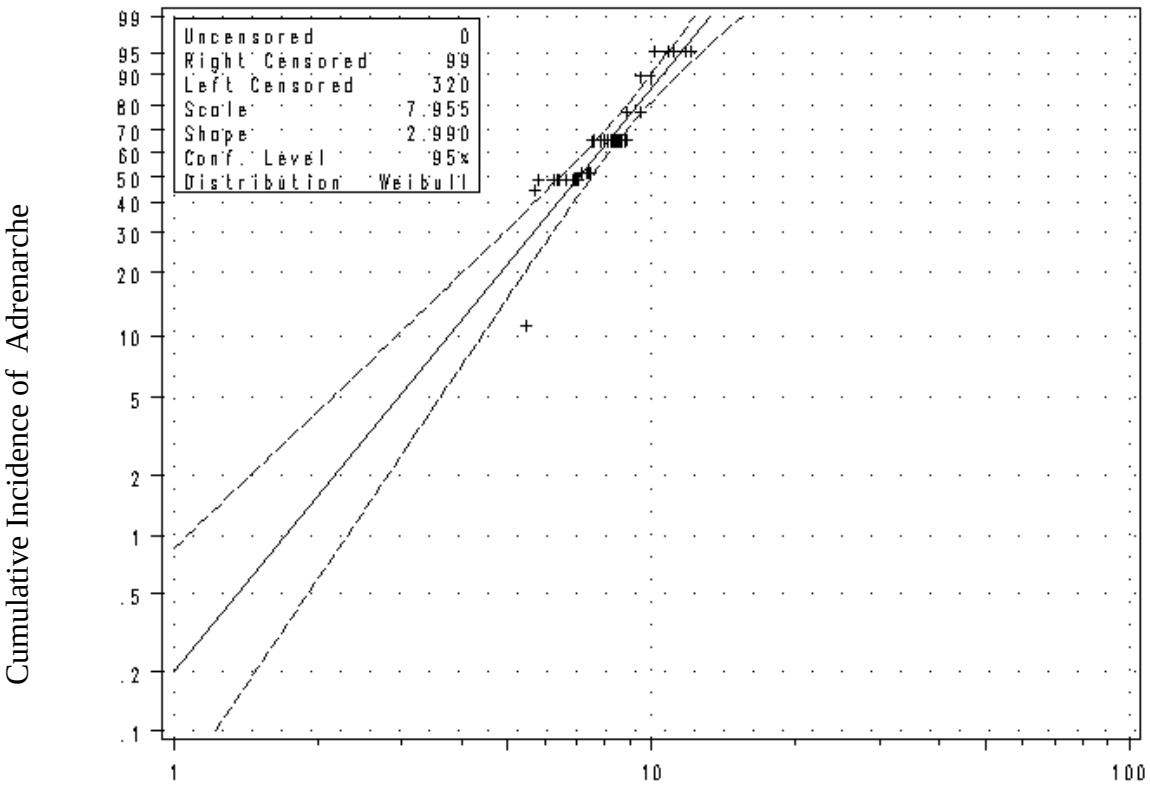


Figure B: Comparison of Weibull and Non-Parametric Models of Thelarche

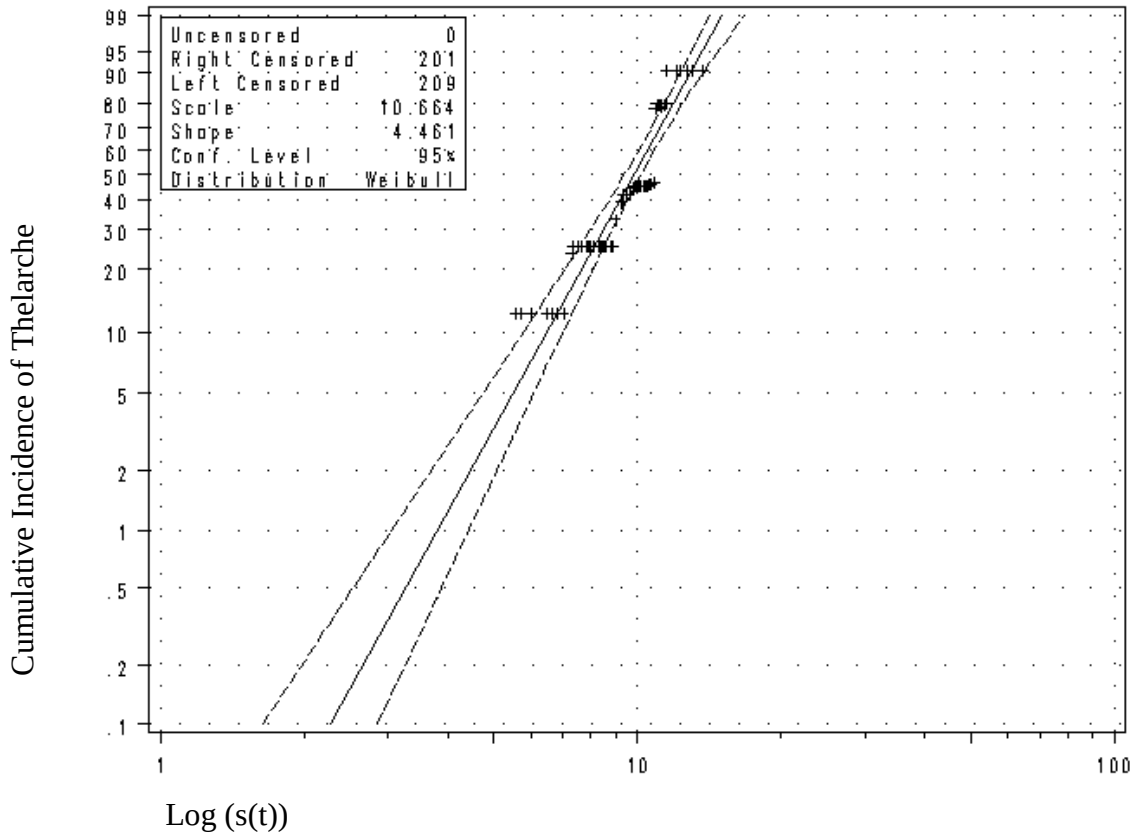


Figure C: Comparison of Weibull and Non-Parametric Models of Pubarche

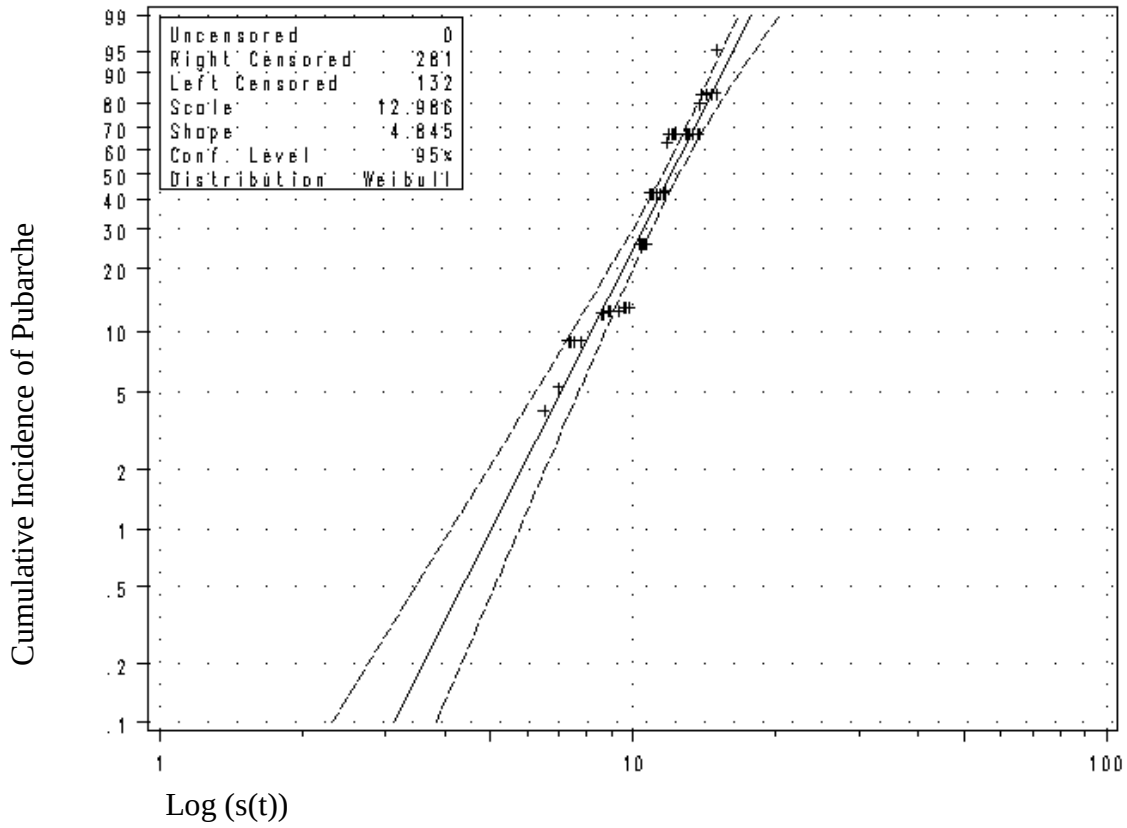
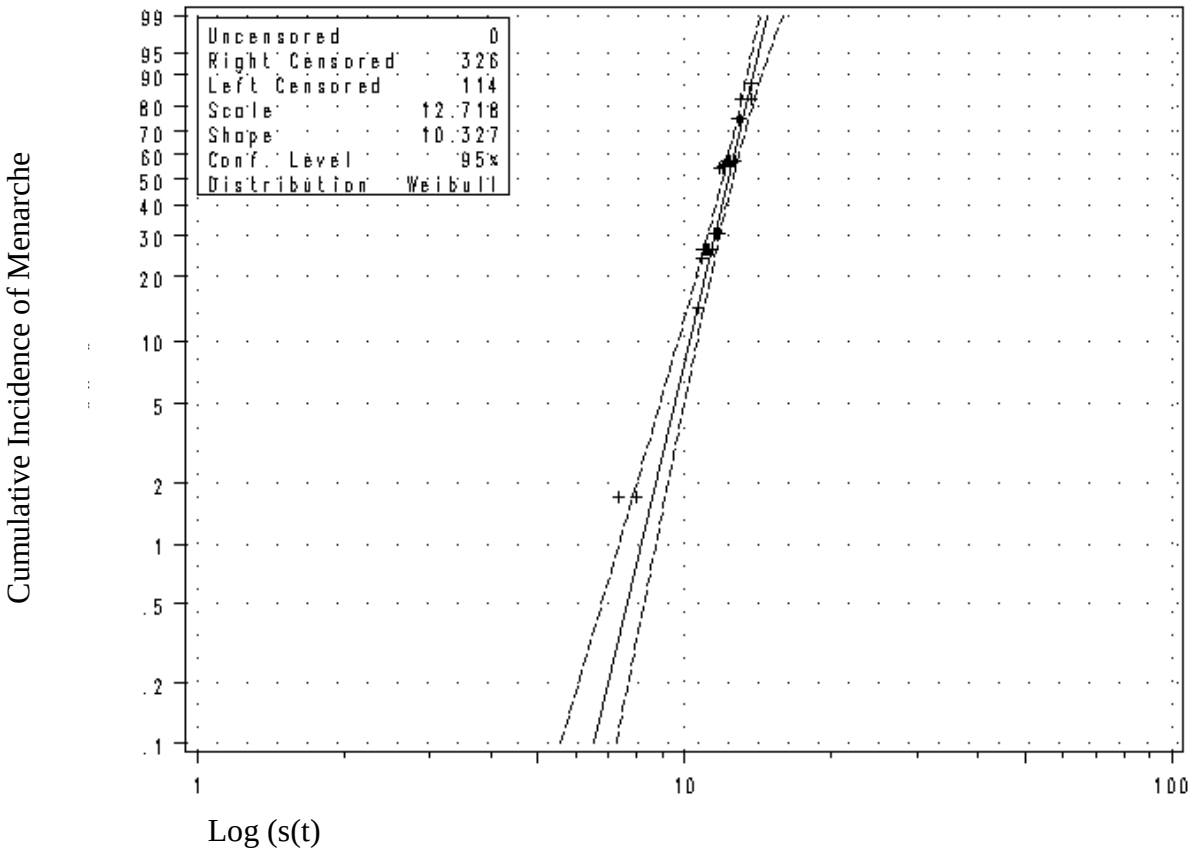


Figure D: Comparison of Weibull and Non-Parametric Models of Menarche



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